ENHANCEMENT IN STABILITYAND IN-VIVO BIOAVAILABILITY OF RIFAMPICIN ALONE AND IN COMBINATION WITH ISONIAZIDUSING ASCORBIC ACID

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by
REG. NO: 26105407

Under the Guidance of

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DEDICATED TO MY PARENTS AND FRIENDS

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P. Tejaswi

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CONTENTS

1. ABSTRACT

Previous study shows (or) reveals that ascorbic acid addition to plasma can prevent the decomposition of the rifampicin. The present study was aimed to investigate the influence of ascorbic acid in different concentrations on the stability and also on the pharmacokinetics of rifampicin in rabbits at p^H1.2 medium. Rifampicin alone and in combination with isoniazid in the presence of ascorbic acid in different ratios (125mg, 250mg,500mg) were used for this study. Percent degradation of rifampicin was significantly reduced by addition of ascorbic acid in pH 1.2 medium and the effect was found to be dependent up on the concentration of ascorbic acid. The study was extended in animal model and it was observed that all pharmacokinetic parameters such as ka, ke, vd t1/2, $C_{max,}$ T_{max}, AUC $_{(0-12)}$, AUC of rifampicin was significantly influenced by ascorbic acid as compared to that of rifampicin alone and in combination with isoniazid Ka, ke,vd, $C_{max,}$ $AUC_{(0-12)}$ and AUC $_{(0-\infty)}$ were increased with increase in ascorbic acid concentration; however, Tmax, T1/2 of rifampicin was shown decreased with increase in ascorbic acid concentration. The results conclusively shows that in presence of isoniazid the absorption of rifampicin was decreased, where as ascorbic acid addition to rifampicin can improve the bioavailability of rifampicin.

INTRODUCTION

2. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. It is the world's second commonest cause of death from infectious disease after HIV/AIDS; approximately one third of the world population is suffering with this disease. TB has been declared a public health emergency by the WORLD HEALTH ORGANIZATION (WHO). A control programmed reaches the WHO targets of 70% case detection and 85% cure. Without greater effort to control tuberculosis, the annual incidence of the disease is expected to increase by 41% (21-61) between 1998 and 2020 (from 7.4million to 10.6 million cases per year).Achievement of WHO targets by 2010would prevent 23% (15-30) or 48 million cases by 2020.[1]

The anti-TB drugs have been categorized into two types. Namely, first line and second line drugs. First line drugs are Rifampicin (RIF) and Isoniazid (INH), Pyrazinamide (PYZ), Ethamabutol (ETB) and Streptomycin. Second line drugs are Capreomycin, Anamycin, Ethionamide, Para -amino salicylic acid, Cyclosporine, Thiacetazone, Ciprofloxacin, Ofloxacin and Sparfloxacin.[2]

INH eradicates most of the rapidly replicating bacilli in the first 2 weeks of the treatment, together with streptomycin and ETB. Thereafter, RIF and PYZ have an important role in the sterilization of lesions by eradicating organisms. These two drugs are crucial for successful 6-month treatment. RIF kills non-replicating organisms and the high sterilizing effect of PYZ serves to act on semi dormant bacilli not affected by any other anti-TB drugs[3], INH and RIF, these two are most potent TB drugs, kills more than 99% of the tubercular bacilli within 2 months initiation therapy.[4]

Rifampicin is a semi synthetic derivative isolated from *streptomyces mediterranei*[5] and is highly effective on short term course of anti tuberculosis regimens and has an important role in killing the semi dormant tubercle bacilli. Rifampicin is bactericidal and acts on both intra and extra- cellular organisms. Rifampicin acts by inhibiting the bacterial DNA-dependent RNA polymerase, thus stopping the expression of bacterial genes. Hepatotoxicity due to Rifampicin has been reported.[6]

Rifampicin, Isoniazid, Pyrazinamide, Ethambutol were earlier prescribed as a separate formulation in TB treatment. To increase the patient compliance, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATID) encourage the use of fixed dose combinations (FDC) tablets that ensures ingestion of all the components therapy. By facilitating the delivery of correct drug doses, FDC are expected to reduce the risk of emergency of drug resistant TB.

After oral administration of Rifampicin on empty stomach, the absorption is rapid with a single 600mg dose peak level is achieved within 2-3hours after administration .Food interferes with its absorption[7]. Almost 85% of the drug gets bound to serum proteins and it is metabolized in the liver to an active metabolite, deacetylrifampin and undergoes enterohepatic recycling. A mean RIF maximal serum concentration (Cmax) of 10.54± 3.18µg/ml the time which it is occurred (Tmax) OF 2.42±1.32hrs, and the (AUC0-∞) of 57.15±13.41µg.h.ml on fasting conditions.[8] The previous study indicates that Rifampicin alone was degrade by 12.4% in acidic medium in 1hr to 3-formylrifampicin and in the presence of Isoniazid the degradation is increased to 21.5% and also indicated that the degradation of Rifampicin to 3-formylrifampicin is almost more than the two times faster in the presence of Isoniazid than that of the Rifampicin alone.[9]

Though FDC of RIF, INH, PYZ,ETB is advocated to combat multi drug resistance TB induced by separate formulations of these drugs, degradation of Rifampicin by the influence of Isoniazid resulting poor bioavailability of Rifampicin is a major concern for effective control of TB. Rifampicin is primarily absorbed from the stomach where as INH absorbed from intestine. Therefore formulations were developed that release RIF in the stomach and INH in the intestine to prevent degradation of Rifampicin by the Isoniazid in the stomach.

Earlier study shows that ascorbic acid administration prevents the degradation of RIF. Another study reports that RIF oxides in solution to form rifampicin quinone and the addition of ascorbic acid slow down this oxidation .[10]Use of rifampicin (1000mg/day) is also recommended in tuberculosis patients.[11]

Thus RIF appears the best choice for TB, however, its poor bioavailability from FDC due to degradation in the stomach in the presence or absence of INH following oral administration still remains a concern for effective management of TB associated with bacterial resistance. Hence development of any method that would standardize RIF against degradation in the stomach will be therapeutically beneficial. So in the present study an attempt was made to investigate the influence of ascorbic acid, an antioxidant and improving the pharmacokinetics of rifampicin following oral administration. The outcome of the study may provide an in-sight into adjusting the dose regimen of rifampicin with improved bioavailability for effective management of tuberculosis.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Tuberculosis is believed to claim 2 million lives a year all over the world. Countries with poor healthcare systems suffer most. Emergency of multi drug resistant (MDR) strains of *M. tuberculosis* and a co-infection with AIDS prompted WHO in March 1993 to declare tuberculosis as Global Health Emergency (Annon, 1997).

Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PZ), and Ethambutol (ETB) are the drugs of choice for treating tuberculosis. Fixed Dose Combinations (FDC) of two, three (or) four drugs is a preferred dosage form for better patient compliance, efficient reduction in viable bacterial population and minimizing development of resistance to anti-tubercular drugs.

Epidemiology of TB

In 1882 Robert Koch made the land mark discovery that TB is caused by an infectious agent *Mycobacterium tuberculosis.* Although demystifying, Koch's finding introduced the possibility that anti-microbial agent could be developed to combat this age-old scourge[12]. Today despite the availability of effective anti-tuberculosis therapy for 50 years. TB remains a major health problem. As the rates of TB infection have fallen dramatically in industrialized countries in the past century, resource poor countries now bear over 90% of all cases globally. In fact, there are more cases of TB today than ever recorded.

Global incidence and prevalence

The WHO estimates that approximately one third of global community is infected with TB[13]. In 2000 an estimated 2-9 million incident cases and approximately 3 million deaths are occurred worldwide due to TB. After the immune deficiency virus (HIV)/AIDS, TB is the second most common cause of death due an infectious disease, and current trends suggest that TB will still be among 10 leading causes of global disease burden in the year of 2020[14].

The global distribution of TB cases is skewed heavily toward low-income and emerging economies. The highest prevalence of cases is in Asia, where China, India, Bangladesh, Indonesia, and Pakistan collectively make up over 50% of the global burden. Africa, have the highest incidence rate of TB, with approximately 83 and 290 per 10000, respectively. TB cases occur predominantly (approximately 6million of the 8 million) in the economically most productive 15-49 year old age group [15]our understanding of TB epidemiology and the efficacy of control activities have been complicated by the emergency of drug-resistant bacilli and by the synergism of TB with HIV infection.

Off the 33.2 million persons infected with Human Immuno Deficiency virus (HIV) one third are estimated to also be infected with *Mycobacterium tuberculosis*. In 2008, there were an estimated 1.4 million new cases of TB among persons with HIV infection, and TB accounted for 26%of AIDS related deaths.[16]

The WHO starts a control programme, for effective control of TB, it reaches 70% case detection and 85% cure would reduce the incidence rate by 11% per year and the death rate 12%per year without the greater effort to control tuberculosis, the annual incidence of the disease is expected to increase by 41% between 1998 and 2020.

TB remains a prominent one in international statistics of ill health mainly because it kills young ones. More than 80% of the burden of TB, as measured in terms of disability adjusted life years lost, is due to premature death rather than illness. About 1.7 million people died of TB in 2004, including 264000 patients who are co-infected with HIV.[17]

Effective control of TB requires an understanding the epidemiology of the disease. The success in reducing the TB burden reflects several factors, including improved public health efforts, physician and patient education, infection control measures, and the use of directly observed therapy (DOT). Future efforts to control the TB will require vigilant public health efforts, improving education of patients and health care personnel and assuring adequate treatment and prophylactic regimens among infected individuals.[18]

Drugs used to treat TB

TB drugs are classified into two categories, namely first line drugs and second line drugs. First line drugs are Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PYZ) and Ethambutol (ETB) and streptomycin. Second line drugs are Capreomycin, Anamycin, Ethionamide, Para amino salicylic acid, Cyclosporine, Thiacteazone, Ciprofloxacin, Levofloxacin, Ofloxacin, and Saprofloxacin.[19]

TB drugs in development.

Gatifloxacin

Gatifloxacin is a fluroquinolone; it blocks the bacterial DNA gyrase, thereby preventing chromosomal replication. Gatifloxacin is currently undergoing phase three clinical trials[20].

OPC-67683

POC67683 is a nitromidazo-oxazole, it inhibits the cell wall synthesis. Otsuka Pharmaceuticals (Japan) are conducting the phase two clinical trails. It is effective against MDR-TB and has no cross resistance with first line TB therapy.[21]

Moxifloxacin

Mechanism of action of Moxifloxacin is a broad spectrum 8- methoxy, fluroquinolone with activity against both gram positive and gram negative bacteria. It inhibits bacterial DNA gyrase as an enzyme that is essential for the maintaience of DNA super coils, which is necessary for chromosomal replication. It is undergoing on phase two and phase three clinical trails.[22]

Pyrroles LL3858

Pyrrole LL3858 has potency against drug sensitive TB strains. Phase one clinical trials are conducting by Lupin Limited (India).[23]

PA-824

PA-824t is a nitroimidazo-oxazole. It inhibits the protein synthesis and lipid synthesis also, currently it is under phase one clinical trials, It is more efficient than Isoniazid and lesser efficient than the Rifampicin.[24]

Rifampicin and its bioavailability

The problem with rifampicin is its poor bioavailability poor bioavailability from Fixed Dose Combinations. Especially in the presence of Isoniazid the decomposition of rifampicin is increases under acidic conditions[25]. The bioavailability of rifampicin reduces when it is co-administered with anta acids like aluminum hydroxide, sodium bicarbonate.[26]

Rifampicin is readily absorbed from the gastrointestinal tract. Nitti et al showed that the pharmacokinetic parameters after intravenous infusion do not differ significantly from those after oral administration of the same doses. Loss et al reported an absolute BA (bioavailability) of 93% after a single oral and intravenous dose of Rifampicin at the beginning of the treatment of six adult patients, decreasing to under70% after repeated dosage due to self induction of metabolizing enzymes by Rifampicin. Rifampicin was reported to show dose dependent absorption, probably due to saturation of efflux systems in the small intestine. Analysis of the absolute BA of Rifampicin in a pediatric revealed that the BA of a freshly prepared oral suspension containing 324 mg/m^2 Rifampicin was only about 50 Rifampicin was only about 50 ±22% of an intravenous dose of 287mg/m^2. Malabsorption of Rifampicin was reported to be common in undernourished patients and patients with AIDS. The C_{max} after oral administration of 600mg of Rifampicin averages from about 8-20µg/ml. C_{max} values in health volunteers, patients with TB and in children can vary widely from individual to individual. A plasma protein binding of 80-91% has

been reported. Most of the unbound fraction is not ionized and diffuses freely in to most tissues. The main metabolic pathway is deacetylation in the liver and the metabolite is excreted via biliary pathway but also renally. With in 24 hours about 3-30% of single oral dose is recovered in the feces. The antibiotics shows dose dependent elimination kinetics.[27]

Degradation of rifampicin

Degradation studies were performed in 0.1N HCL at 37^0C in (USP dissolution apparatus) in absence (or) presence of INH. Both RIF and INH were analyzed. The degradation of RIF was increased approximately three fold in the presence of INH. INH itself was degraded to lesser extent.[28, 29]

Rifampicin is well absorbed from the stomach at pH 1.2 due its solubility. Isoniazid is poorly absorbed from the stomach, but it is well absorbed from the intestine[30]. Degradation of rifampicin is increases when it is co-administered with isoniazid, but isoniazid disappearance was not influenced by rifampicin.

Methods to prevent the degradation of Rifampicin

Rifampicin is degrades rapidly in plasma at ambient temperature, and 54% loss was observed within 8hrs. This degradation is effectively decreases by adding the ascorbic acid. Rifampicin oxidizes in to solution form rifampicin quinine. Ascorbic acid is added to that solutions forms of Rifampicin to slows down the oxidation and no degradation was observed in thawed samples up to 9hrs. [1]

Contact between the Rifampicin and Isoniazid can decrease the degradation of the Rifampicin. Delay of the Rifampicin release in the acidic medium was achieved by preparing the Rifampicin-Sodium Laurly Sulphate mixture in the ratio of 1;1 by co-grinding method which is a relatively simple and effective method. Thus this approach is beneficial for the segregation of release pattern of Rifampicin in alkaline environment and Isoniazid in the acidic environment of the GI tract, which will lead to decrease the degradation of Rifampicin alone and in combination with Isoniazid.[30]

Back ground of the study

The literature review indicates that only few approaches including nanoparticles have been attempted to prevent (or) minimize the degradation of rifampicin with improved bioavailability. Ascorbic acid, an antioxidant has been reported for preventing degradation of rifampicin in the plasma sample. Additionally, rifampicin administration (1000mg/day) is also recommended in patients infected with tuberculosis. To our knowledge, no study is available on the effect of ascorbic acid on the stability of rifampicin in animal model. Therefore, the present study attempted to investigate the same.

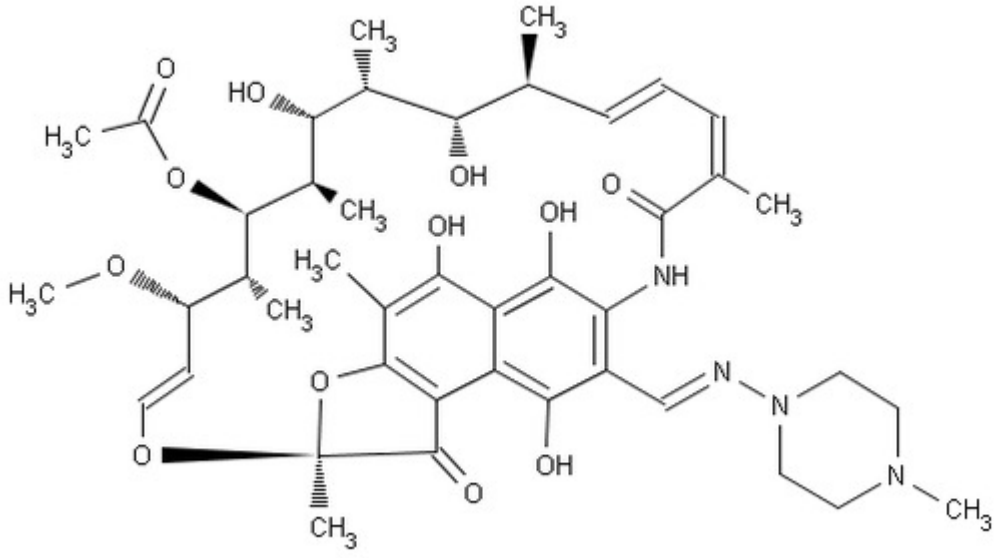
PROFILES

4. RIFAMPICIN

Rifampicin

It is a semi-synthetic derivative of Rifamycin B isolated from *Streptomyces mediterranei*.

Structure:



Empirical formula:

$C_4H_{58}N_4O_{12.}$

Chemical name:

3-[[(4-methyi 1-piperazinyl)imino]methyl]-5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptemethyl-8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,.13]trienimino)naphtho[2,1-b]furan-1,11-(2H)-dione21 acetate[13292-46-1].

Physico- chemical properties.

Colour:

Red- orange.

Melting point:

138- 188.

Odour:

Odourless.

Solubility:

Slightly soluble in water, Soluble in dilute acidic solutions.

Molecular weight:

822.94.

Mechanism of action[31]

Rifampicin inhibits the bacteria RNA polymerase, the enzyme is responsible for DNA transcription, by forming a stable drug enzyme complex with a binding constant of 10-9M at 37^0C. The corresponding mammalian enzymes are not infected by Rifampicin. Bacterial resistance to Rifampicin is caused by mutation leading to a change in the structure of the β sub unit of RNA polymerase. Such resistance is not an all-or- nothing phenomenon rather, a large number of RNA polymerase with various degrees of sensitivity to Rifampicin have been found. No strict co-relation exists between enzyme sensitivity and MIC values, since inhibition of RNA synthesis does not always show up to the same extent in the two different test systems used for the determination of these values.

Formulations of Rifampicin in the market:

Rifampicin capsules 150 mg, 300 mg, 450 mg, 600 mg.

Routes of entry:

Oral:

This is the most frequent route of administration.

Dermal:

Not applicable.

Inhalation:

Not applicable.

Parental:

Rifampicin may be given intravenously.

Eye:

Used to treat ocular Chlamydia infection.

Pharmacokinetics

Absorption

Rifampicin is well absorbed from the gastrointestinal tract. After a dose of 600mg orally given at least half an hour before breakfast, the peak plasma level is achieved with in 2-3 hrs. Food interferes the absorption of the Rifampicin.

Distribution

It is widely distributed throughout the body. Almost 85% of the drug gets bound to serum proteins. It crosses the placenta, when the meninges are inflamed; the rifampicin enters in to the cerebrospinal fluid. It is lipid soluble. The effective concentrations are present in the lungs, pleural fluid, bile, liver, and urine .It is also distributed in to breast milk, The apparent volume of distribution is 0.93-0.6L/kg.

Metabolism

The main metabolic pathway is deacetylation in the liver. The API itself and its deacetylated metabolite are mainly excreted via the biliary pathway but also renally. Rifampicin undergoes enterohepatic circulation but its metabolite does not.

Elimination by route of exposure

Rifampicin metabolite is excreted in the bile and also in the urine. Approximately 50% of the dose is eliminated with in 24hrs and 6-30% of the dose is eliminated in unchanged form in the urine, 15% is eliminated as active metabolite. Approximately 43-60% of oral dose is eliminated in the feces.[27]

Interactions[7]

1. If Rifampicin is co-administered with antacids containing aluminum hydroxide, it reduces the bioavailability of Rifampicin.

2. The Para amino salicylic acid granules and placebo granules significantly decreased the absorption of Rifampicin.

3. When Rifampicin is co-administered with oral contraceptive pills, it may cause some menstrual disorders.

4. Rifampicin can decrease the Vitamin D absorption because of induction of enzyme activity.

5. Food lowers the peak blood levels because of its interference with absorption of Rifampicin.

6. The drug induces hepatic microsomal enzymes and thus could cause increased metabolism of several drugs like Hydrocortisone, Verapamil, Phenytoin, Theophylline.

7. Rifampicin and Isoniazid interaction leads to hepatotoxicity.

8. Alcohol intake with Rifampicin increases the risk of hepatotoxicity.

Adverse effects

1. Skin rashes.

2. Diarrhea.

3. Ataxia.

4. Dizziness.

5. Hepatitis.

6. Leucopenia.[7]

Toxicity[7]

1. Renal failure.

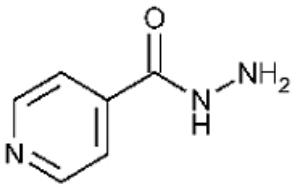
2. Hemolysis.

3. Thrombocytopenia.

ISONIAZID

Isoniazid is a synthetic derivative of nicotinic acid with anti-mycobacterial properties, this is active against actively growing myco-bacteria because it is a pro-drug.

Structure:



Chemical name:

Nicotinic acid hydrazide.

Molecular formula:

$C_6H_7N_3O$.

Molecular weight:

137.14.

Physical and chemical properties

Colour:

White crystalline powder.

Solubility:

Soluble in water, slightly soluble in chloroform.

Melting point:

170-173^{0}C.

Mechanism of action

Isoniazid is a pro drug. It is activated by the mycobacterial catalase- peroxidase to an active compound which inhibits the synthesis of mycolic acid, an important constituent of the mycobacterial cell wall. In addition, the drug inhibits the same catalase-peroxidase and makes the organism susceptible to oxidative mechanisms.

Formulations of Isoniazid in the market:

Isoniazid tablet 100mg, and 300mg.

Routes of entry:

Oral:

This is the most frequent route of intoxication because the drug is usually administered orally.

Inhalation:

Not applicable.

Dermal:

Not applicable.

Pharmacokinetics[32]

Absorption

Isoniazid is rapidly and almost completely absorbed from the gastro-intestinal tract. Peak plasma concentrations are reached within 1-2 hrs after oral administration.

Distribution

Isoniazid is distributed in to all body tissues and fluids. It penetrates well into the saliva, milk, pleural fluids; It can cross the placental barrier freely.

Biological half-life by route of exposure

The plasma half life in patients with normal renal and hepatic function is 1-4hrs.

Metabolism

The major route of Isoniazid metabolism is hepatic acetylation by N-acetyl transferase which produces acetyl isoniazid. The rate of acetylation is genetically determined. Acetyl Isoniazid is further hydrolyzed to isonicotinic acid and acetyl hydrazine both of which are excreted in urine.

Elimination of route of exposure

Approximately 75-95% of orally administered INH is excreted in urine within first 24hrs, mainly acetyl isoniazid and isonicotinic acid. In addition, small quantities are excreted after conjugation with glycine and as isonicotonyl hydrazone.

Interactions

1. Amino salicylic acid, Procainamide, Propranolol increase INH serum levels by reduction of the acetylation.

2. Aluminum containing anta acids decrease the gastrointestinal absorption of INH.

3. Pyrazinamide decreases the serum levels of INH.

4. Food decreases the absorption of INH.

5. Drugs which decreases the serum levels when they are used with INH.

Cyclosporine, Folic acid, ketoconazole, and Verapamil.

6. Drugs which increases the serum levels when they are used with INH

Phenytoin, Diazepam.

Adverse effects

1. Nausea.

2. Loss of appetite.

3. Dark urine.

4. Abdominal pain.

5. Allergy.

6. Liver damage.

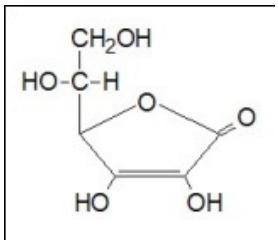
7. Dryness of the mouth.

ASCORBIC ACID

Ascorbic acid

Ascorbic acid is a form of Vitamin C. Ascorbic acid supplements are vital to the health and well being of individuals. It is not naturally produced in the body , It is found in citrus fruits like oranges, grapes, and lemons.

Structure:



Chemical name:

L-ascorbic acid.

Empirical formula:

$C_6H_8O_6$.

Molecular weight:

176.1.

Melting point:

190^0C.

Colour:

White to slightly yellowish colour.

Taste:

Slightly acidic.

Solubility:

Freely soluble in water, sparingly soluble in alcohol, insoluble in chloroform.

Mechanism of action

Ascorbic acid has potent anti-oxidant properties, meaning is able to reduce damage caused by oxidizing chemicals, such as free radicals. These oxidizing chemicals called as reactive species (ROS), are the normal by products of the cellular reactions takes place in our body. Oxidizing agents are very un stable and damage our cells by reacting with important molecules and changing how they function. Ascorbic acid reduces this damage by directly binding to oxidizing chemicals and converting them to less harmful molecules. Reducing oxidative damage have many benefits for our body, including reducing cancer, and heart diseases.

Dosage and administration[33]

Ascorbic acid is usually administered orally. When the oral administration is not feasible, the drug may be administered intra-muscular, and intra-venous. When it is given by parental route, the utilization of Ascorbic acid is more. The average protective dose of this one is for adults are 70-510mg daily. In the presence of scurvy, doses of 300mg-1gm daily recommended.

Functions

1. Facilitates the growth and repair of the tissue.

2. It participates in the formation of collagen which is an important connective tissue protein in skin, bones, teeth, and blood vessels.

3. Improves the iron and calcium absorption.

4. Participates in the transformation of cholesterol in to bile salts.

5. Prevents the antihistamine activity.

6. Helps in healing of wounds, fractures, and hamorrhages.[34]

7. Ascorbic acid is a powerful anti-oxidant that can protect our body from the effects of free radicals.

8. Additionally it reduces the susceptibility of infections.

9. Helps in strengthening our immune system.

Uses

1. It has been used as a means of either curing (or) preventing the common cold.

2. It is used to treat tyrosinemia disease.

3. Used to treat the infections like bladder and prostate.

4. Used for hardening of the arteries.

5. Used for preventing the cataracts.

6. Used in adjuvant cancer.[35]

7. Treating lead, mercury poisoning.

8. Reducing leg cramps during pregnancy.

9 .In iron deficiency anemia.[36, 37]

10. Treating loose teeth.[38]

Toxicity

1. Gastrointestinal disturbances like stomach cramps, nausea, and may increase the risk of developing the stones in kidneys.[39]

2. Destruction of red blood cells.[40]

3. Fluctuations in the blood pressure.

4. Irregular breathing.

5. Excessive chewing of ascorbic acid (Vitamin C) may leads to jaundice.

AIM AND OBJECTIVE

5. AIM AND OBJECTIVE

To investigate the effect of ascorbic acid on the stability and pharmacokinetics of rifampicin alone and in combination with Isoniazid in rabbits. The following objectives were examined.

In-vitro dissolution stability studies on;

a) Rifampicin (300mg).
b) Rifampicin (300mg) +Isoniazid (150mg)
c) Rifampicin (300mg) +Isoniazid (150mg) +Ascorbic acid (125mg).
d) Rifampicin (300mg) +Isoniazid (150mg) +Ascorbic acid (250mg).
e) Rifampicin (300mg) +Isoniazid (150mg) +Ascorbic acid (500mg).
f) Rifampicin (300mg) +Ascorbic acid (125mg).
g) Rifampicin (300mg) +Ascorbic acid (250mg).
h) Rifampicin (300mg) +Ascorbic acid (500mg).

In-vivo pharmacokinetic study:

1. Rifampicin. (21.4mg/kg).

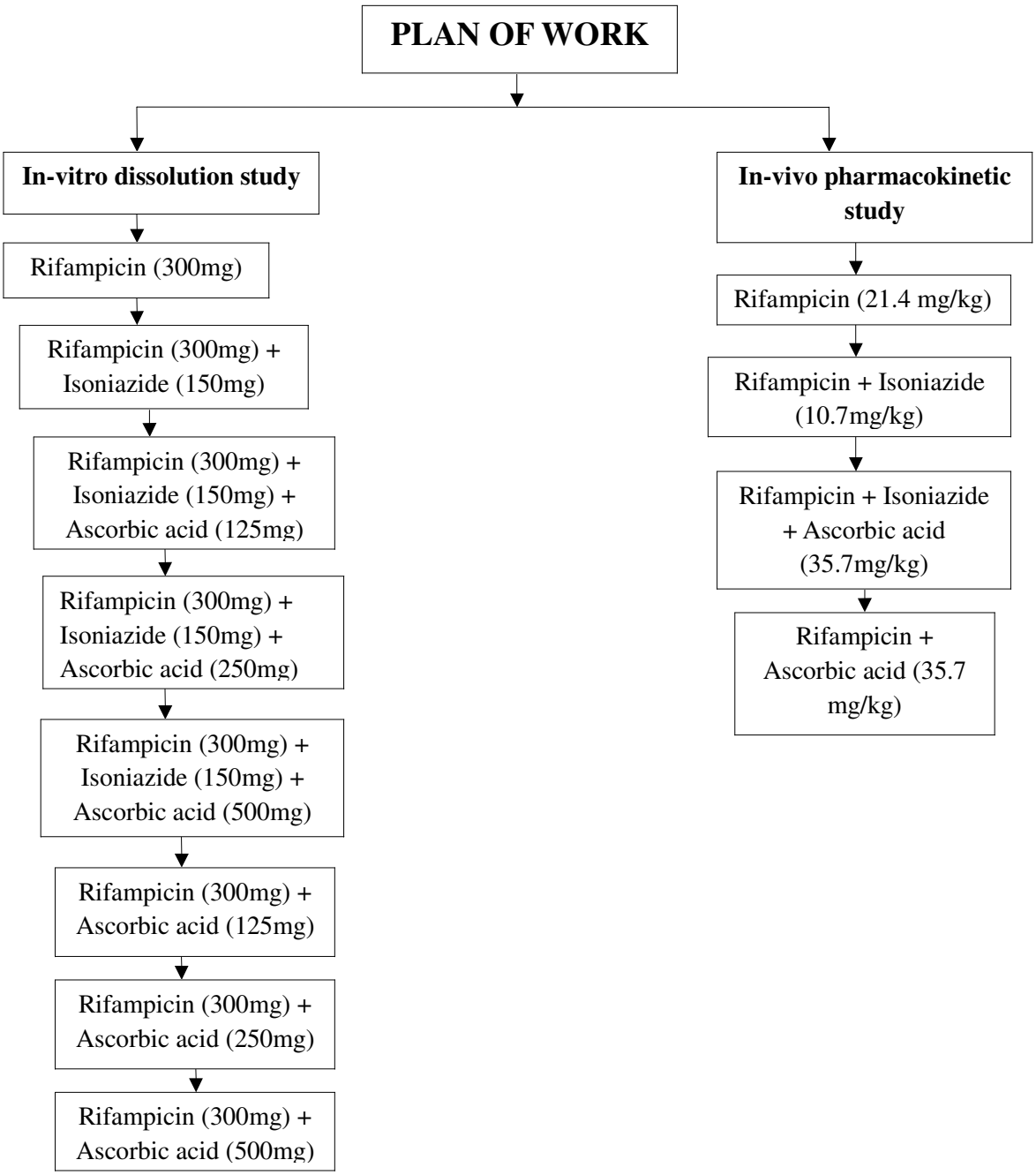
2. Rifampicin (21.4mg/kg) +Isoniazid (10.7mg/kg).

3. Rifampicin (21.4mg/kg) +Isoniazid (10.7mg/kg) +Ascorbic acid (35.7mg/kg)(Best formulation of in-vitro).

4. Rifampicin (21.4mg/kg) +Ascorbic acid (35.7mg/kg) (Best formulation of in-vitro).

PLAN OF WORK

6. PLAN OF WORK



METHODOLGY

7. METHOLODGY

In-vitro dissolution stability study

Drugs:

Rifampicin pure a gift sample and isoniazid was obtained from Astha Laboratories Pvt. Ltd**,** Hyderabad, Andhra Pradesh, India. Ascorbic acid was obtained from Qualigens Fine Chemicals, Mumbai.

Chemicals:

KCl from Loba Cheme Pvt Ltd, Mumbai.

HCl from Loba Cheme Pvt Ltd, Mumbai.

Preparation of pH 1.2

50ml 0f 0.2M KCl was mixed with 85ml of 0.2M HCl and make up to 900ml with water.

In-vitro dissolution stability study of RIF in pH 1.2 solution

Dissolution profile of pure rifampicin(300mg), rifampicin(300mg) +isoniazid (150mg) rifampicin (300mg)+isoniazid(150mg) +ascorbic acid in varying concentrations (125mg, 250mg 500mg)and rifampicin +ascorbic acid in varying concentrations (125mg, 250mg 500mg) were evaluated according to the method described in USFAD. Preparer 900ml (0.1N HCL) of the dissolution medium of pH 1.2 buffer and pour it in to the vessel and temperature of medium was maintained at 37±0.2°C . The sample was placed in the medium and the dissolution was performed at 100rpm. 10ml of samples were withdrawn at 0, 15, 30, 45, and 60 min and equivalent amount of dissolution medium were added to maintain sink conditions . The obtained samples analyzed by UV spectrophotometer at 475nm. The experiment were carried out in triplicate and mean values and SD were recorded and the percentage degradation was calculated by using the given formula.[2]

% Degradation loss was calculated by using formula

$$\frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} X100$$

In-vivo pharmacokinetic study

Animals:

A white New Zealand rabbits weighing 1.5 to 2.5kg were obtained from Swamy Vivekananda College of pharmacy animal house .The animals were feed with cabbage and water. They were maintained in standard laboratory conditions 21±2 ^{0}C and relative humidity of 55-60%. The animals were overnight fasted before the experiment. The study protocol was approved by the Institutional Animal Ethical Committee and the protocol number is SVCP/IAEC/M.Pharm/03/2011.

Drugs:

Rifampicin and Isoniazid were obtained from Astha Laboratories Pvt. Ltd, Hyderabad, Andhra Pradesh, India. Ascorbic acid was obtained from Qualigens Fine Chemicals, Mumbai.

Chemicals:

1% CMC from Loba Cheme Pvt Ltd, Mumbai.

95% v/v alcohol.

Requirements:

Cotton.

Surgical blade.

26G needle.

Blood collecting tubes (EDTA tubes).

Plasma sample collecting tubes.

Sex:

Both Male/Female.

No. of animals:

12.

Animal dose:

Rifampicin:

21.4mg/kg p.o.

Isoniazid:

10.7mg/kg p.o.

Ascorbic acid:

35.7mg/kg p.o.

Procedure for collection of blood:

The rabbit was placed in a restrainer. The hair present on the ear was shaved smoothly with blade without disturbing the blood vessels. Ear was cleaned with 95% v/v alcohol on the collection site and rapid rubbing on the ear to dilate blood vessels which is easily to collect the blood. 2G needle was inserted in to the marginal ear vein to collect the blood from marginal ear vein. After collecting the blood, clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding.[42]

Experimental procedure: Rabbits were classified into 4 different groups each group consisting of 3 animals.

Group1: Control (Rifampicin alone).

Group 2: Rifampicin + Isoniazid.

Group3: Rifampicin+ Isoniazid+ Ascorbic acid.

Group4: Rifampicin+ Ascorbic acid.

Preparation of samples:

The samples were suspended in water using 1%CMC as a suspending agent and used for this study.

Each group of animals (three rabbits) was administered the samples as shown as above through an intra gastric tube after a overnight fasting. Blood samples (1 ml) were collected in to heparinized tubes from the marginal ear vein at 0, 0.5, 1, 2, 4, 6, 9 and 12 h after drug administration and plasma was separated by using centrifugation and stored at -20°C. Samples were by analyzed by high performance liquid chromatography (HPLC).[43]

BIOANALYTICAL WORK

Extraction of rifampicin from plasma:

The mobile phase consisted of acetonitrile 0.05 M sodium citrate buffer adjusted to pH 4.0 with 0.05 M hydrochloric acid (42:58) and pumped at a flow-rate of 2.3 ml/min a ant ambient temperature. Buffer was filtered through an Whatman filter paper. Mobile phase was filtered (cellulose acetate filter diameter 47 mm, pore size 0.45 µm, Sartorius AG. 370700) and sonicated (EYELA-Sonicator) for 12 min.[44]

An aliquot of 200 µl of plasma samples was pipette into an eppendrof's tube of 1.5ml capacity. Acetonitrile (300 µl) was added, vortexes for one minute and micro centrifuged at 10,000 rpm for 5min. then 300 µl of the supernatant was taken into another micro centrifuge tube and vacuum dried in the HETO vacuum centrifuge. The residue obtained was reconstituted in 100 µl of mobile phase. Plasma was filtered through 0.22 µm membrane (13 mm) and 20 µl volumes was injected.[45]

HPLC analyses

Rifampicin was analyzed at ambient temperature on a Kromasil C18 column Phenomenex, UK, 100mm×3.2mm i.d., 3µm particle size) with an ODS Securigard® guard column 4mm×3mm (Phenomenex, Macclesfield, UK). The mobile phase ammonium acetate buffer adjusted to pH 4.0 with glacial acetic acid and acetonitrile was delivered at 0.7ml/min on a gradient program (20-90% acetonitrile over 18 min) by a spectra system P2000 pump. The analytes were detected by a UV detector at 334nm. The injection volume for each sample was 20µl to column.[1]The peak was found at 2.52 min, which was probably the 25-desacetylrifampicin metabolite. HPLC analyses showed that the assay method is linear in the ranges 0.1-1µg/ml for plasma. The chromatograms were recorded according to the "Area normalization method" with the measurement of peak area.[46]

Extraction efficiency

The extraction efficiency was calculated by comparing the peak heights of rifampicin spiked-pooled blank plasma samples with that of respective standard rifampicin samples.

Calibration curve for rifampicin

A calibration curve of rifampicin was obtained by plotting the concentration of rifampicin against the respective spiked-pooled blank plasma samples peak area. Concentration of rifampicin was calculated by using the following equation.

$$Y = MX + C$$

Pharmacokinetic parameters[47, 48]

The pharmacokinetic parameters were calculated for each rabbit of group I, group II, group III and group IV, by the semi logarithmic plot of plasma rifampicin concentration at various intervals. The following pharmacokinetic parameters were calculated:

1. **Elimination rate constant (K_e):** The elimination rate constant was calculated by using the formula.

$$K_e = -2.303 \times \text{slope of extrapolated curve.}$$

2. **Elimination half life ($t_{1/2}$):** $t_{1/2}$ was calculated by the following using the formula.

$$t_{1/2} = 0.693/K_e$$

3. **Absorption rate constant (K_a):** This was calculated by the method of residuals. The log linear portion of the decline phase was back extrapolated for each curve. The plasma concentration along this extrapolated line was C. the observed plasma concentration C was subtracted from the corresponding extrapolated value at each time point. The semi logarithmic plot of residuals (C-C) against time yields a straight line.

$$K_a = -2.303 \times \text{slope of residual line}$$

4. **Absorption half life:** It was calculated using the formula.

5. **$T_{1/2(a)}$ apparent volume of distribution (V_d):** It was calculated by using the following formula.

$$V_d = \frac{K_a F X_0}{(K_a - K_e) \text{ y intercept}}$$

6. **T_{max}** was calculated using the formula.

$$t_{max} = \frac{\ln K_a - \ln K_e}{K_a - K_e}$$

7. **Maximum plasma concentration (C_{max}):** C_{max} was calculated using the formula.

$$C_{max} = \text{Y intercept } (e^{-Ke.\,Tmax} - e^{-Ka.\,Tmax})$$

8. **Area under curve (AUC_{0-12}):** AUC_{0-12} was calculated using the formula.

$$AUC = \frac{F X_0}{V_d \cdot Ke}$$

9. **$AUC_{0-\infty}$** was calculated using the formula.

$$AUC_{0-\infty} = \frac{C_0}{K_e}$$

Statistically analysis:

The data were analyzed. One way ANOVA followed by Tukey's multiple comparison Test with the help of Graph Pad Instat software, version 3.01. ($P<0.05$)considered as significant.

RESULTS AND DISCUSSION

8. RESULTS AND DISCUSSION

IN-VITRO STABILITY STUDY:

Table1.

Calibration curve of pure Rifampicin in p^H1.2

Concentration	Absorbance
0	0
2	0.18
4	0.41
6	0.62
8	0.84
10	1

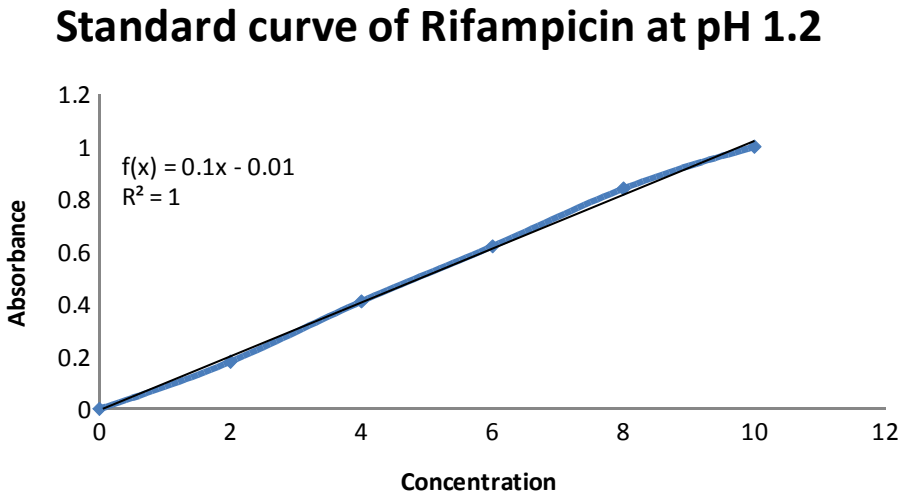


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Figure- 1

Table2.

Calibration curve of Isoniazid at pH1.2

Concentration	Absorbance
0	0
2	0.21
4	0.43
6	0.64
8	0.85
10	0.97

Figure-2

Table3.

Calibration curve of Rifampicin +Isoniazid +Ascorbic acid (125mg)

Concentration	Absorbance
0	0
2	0.17
4	0.37
6	0.56
8	0.74
10	0.9

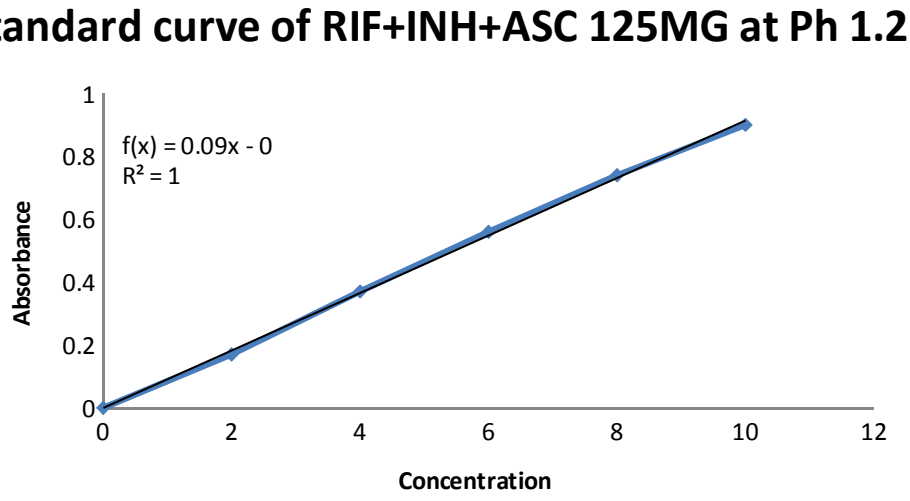


Figure-3

Table4.

Calibration curve of Rifampicin +Isoniazid +Ascorbic acid (250mg)

Concentration	Absorbance
0	0
2	0.2
4	0.43
6	0.63
8	0.84
10	0.99

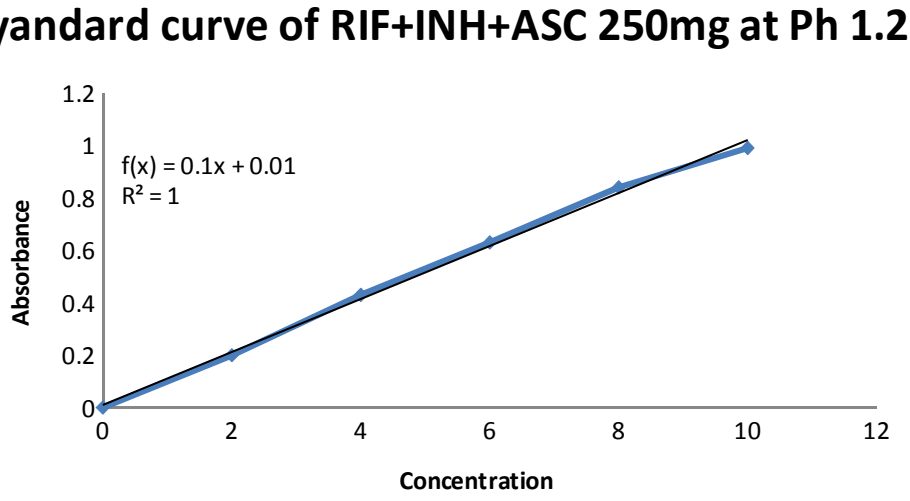


Figure-4

Table5.

Calibration curve of Rifampicin +Isoniazid +Ascorbic acid (500mg)

Concentration(µg)	Absorbance
0	0
2	0.2
4	0.41
6	0.62
8	0.78
10	0.95

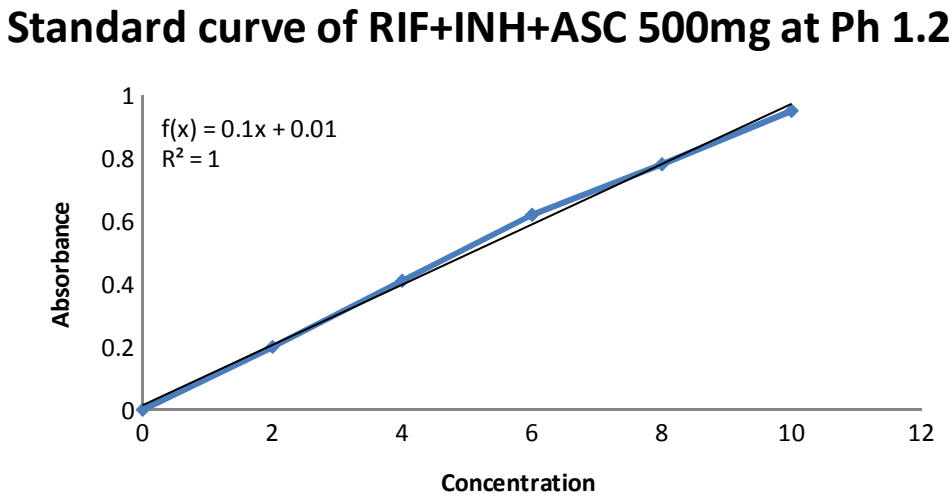


Figure-5

Table6.

Calibration curve of Rifampicin +Ascorbic acid (125mg)

Concentration(µg)	Absorbance
0	0
2	0.21
4	0.42
6	0.64
8	0.86
10	0.99

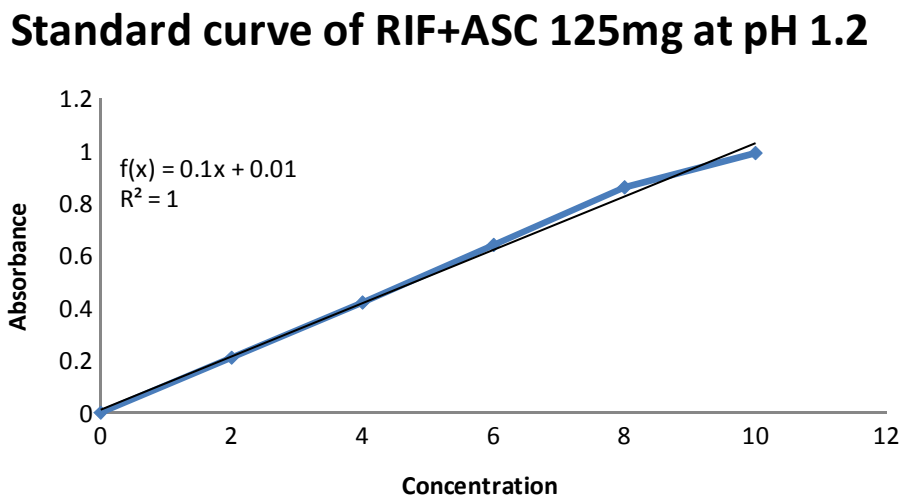


Figure-6

Table7.

Calibration curve of Rifampicin +Isoniazid (250mg)

Concentration (µg)	Absorbance
0	0
2	0.23
4	0.4
6	0.59
8	0.74
10	0.92

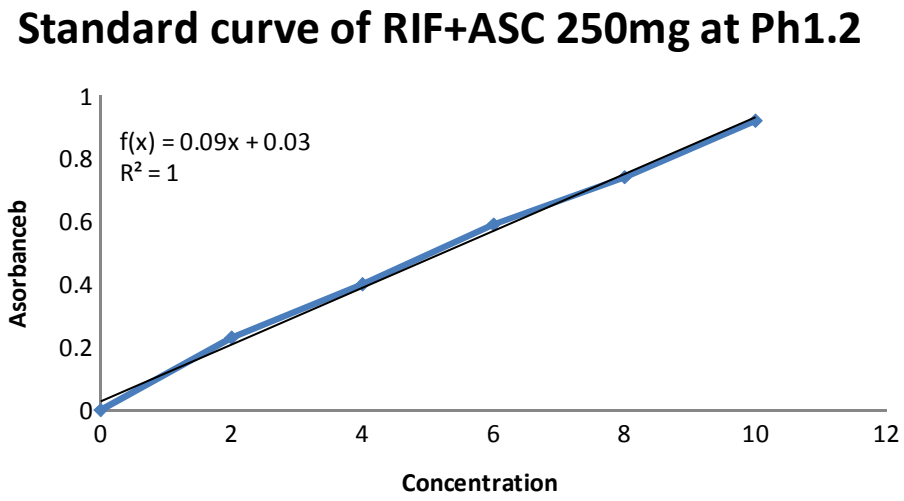


Figure-7

Table 8.

Calibration curve of Rifampicin +Ascorbic acid (500mg)

Concentration(µg)	Absorbance
0	0
2	0.21
4	0.41
6	0.61
8	0.82
10	0.98

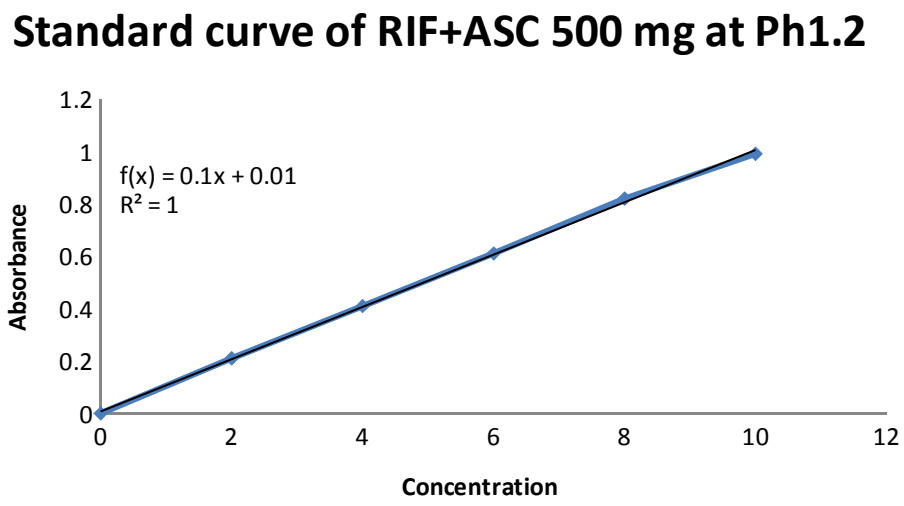


Figure-8

TABLE9.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN ALONE AT PH 1.2

Time(min)	Trial 1	Trial2	Trial3	Cumulative %drug release
0	0	0	0	0
15	30	32.3	35.1	32.4±2.554
30	41	42.5	40.1	41.2±1.212
45	48	47.6	45.1	46.9±1.572
60	56	56.4	59.4	57.2±1.858
				**P<0.01

Table10.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AT PH 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	14	13.9	14.3	14±0.2082
30	18	18.5	19	18.5±0.5
45	21	22	20.5	21.1±0.7638
60	26	25.3	26.7	26±0.7
				**P<0.01

Table11.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (125MG) AT P^H 1.2

Time(min)	Trial1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	40	41.9	42.7	41.5±1.387
30	51	51.7	50	50.9±0.8544
45	59	59.6	60	59.5±0.5033
60	64	63.7	64.8	64.1±0.5686
				***P<0.001

Table12.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (250MG) AT P^H 1.2

Time(min)	Trial 1	Trial2	Trial3	Cumulative %drug release
0	0	0	0	0
15	44	44.3	43.7	44±0.3
30	56	56.7	55.8	56.1±0.4726
45	60	61.1	62	61±1.002
60	69	70	71.3	70.1±1.153
				***P<0.001

Table13.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (500MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	50	50.7	51	50.5±0.5132
30	59	59.3	60	59.4±0.5131
45	63	63.1	62.7	62.9±0.2082
60	74	74.3	75	74.4±0.513
				***P<0.001

Table14.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (125MG) AT P^H 1.2

Time(min)	Trial 1	Trial2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	45	45.3	44.7	45±0.3
30	56	56.2	57	56.4±0.5292
45	61	61.7	60.8	61.1±0.4726
60	68	67.3	68.2	67.8±0.4726
				***P<0.001

Table15.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (250MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	48	48.3	47.7	48±0.3
30	60	61.2	60.3	60.5±0.6245

45	64	65	65.4	64.8±0.7211
60	75	75.3	76	75.4±0.5132
				***P<0.001

Table16.

PERCENTAGE DRUG RELEASE OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (500MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	55	54.9	55.3	55±0.2082
30	64	64.6	63.9	64.1±0.3786
45	68	68.2	69	68.4±0.5292
60	79	78.8	81.2	79.6±1.332
				***P<0.001

COMPARATIVE PERCENTAGE DRUG RELEASE OF

RIFAMPICIN AT PH1.2

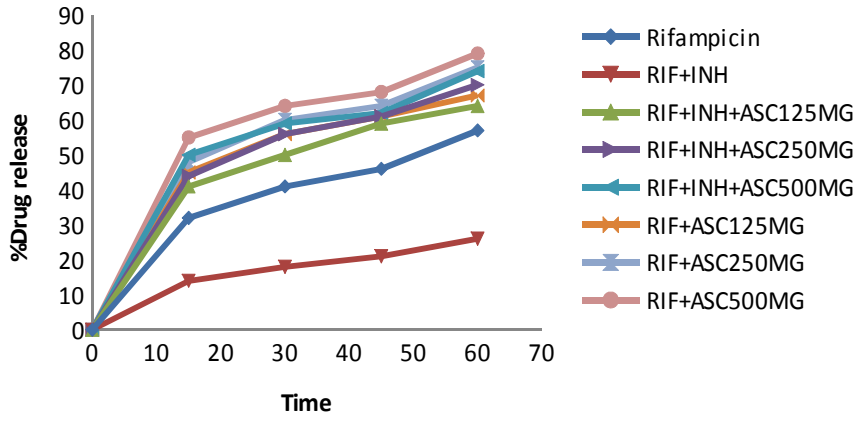


Figure9

Table17.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN ALONE AT PH 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	70	67.7	64.9	67.5±2.554
30	59	57.5	59.9	58.8±1.212
45	52	52.4	54.9	53.1±1.572
60	44	43.6	40.6	42.7±1.858
				***P<0.001

Table18.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AT PH 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	86	86.1	85.7	85.9±0.2082
30	82	81.5	81	81.5±0.5
45	79	78	79.5	78.8±0.7638
60	74	74.7	73.3	74±0.7
				**P<0.01

Table19.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (125MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	60	58.1	57.3	58.4±1.387
30	49	48.3	50	49.1±0.8544

45	41	40.4	40	40.4±0.5033
60	36	36.3	35.2	35.8±0.5686
				***P<0.001

Table20.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (250MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	56	55.7	56.3	56±0.3
30	44	43.3	44.2	43.8±0.4726
45	40	38.9	38	38.9±1.0
60	29	30	28.7	29.2±0.6807
				***P<0.001

Table21.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AND ASCORBIC ACID (500MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	50	49.3	49	49.4±0.5132
30	41	40.7	40	40.5±0.513
45	37	36.9	37.3	37±0.2082
60	26	25.7	25	25.5±0.5132
				***P<0.001

Table22.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (125MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	55	54.7	55.3	55±0.3
30	44	43.8	43.8	43.8±0.1528
45	39	38.3	39.2	38.8±0.4726
60	32	32.7	31.8	32.1±0.472
				***P<0.001

Table23.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (250MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	52	51.7	52.3	52±0.3
30	40	38.8	39.7	39.5±0.6245
45	36	35	34.6	35.2±0.7211
60	25	24.7	24	24.5±0.5132
				***P<0.001

Table24.

PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID (500MG) AT P^H 1.2

Time(min)	Trial 1	Trial 2	Trial 3	Cumulative %drug release
0	0	0	0	0
15	45	45.1	44.7	44.9±0.2082
30	36	35.4	36.1	35.8±0.3786
45	32	31.8	31	31.6±0.5292
60	21	21.2	18.8	20.3±1.332
				***P<0.001

COMPARATIVE PERCENTAGE DRUG DEGRADATION OF RIFAMPICIN AT PH1.2

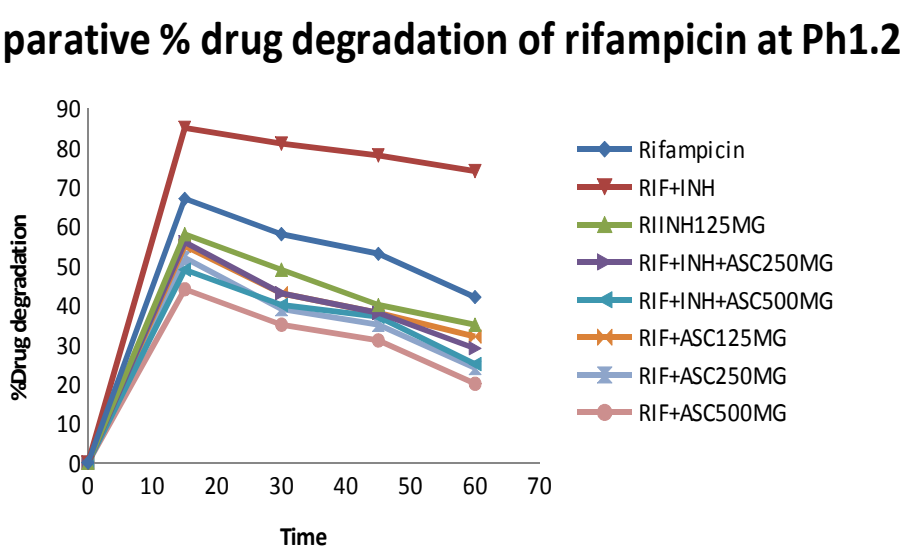
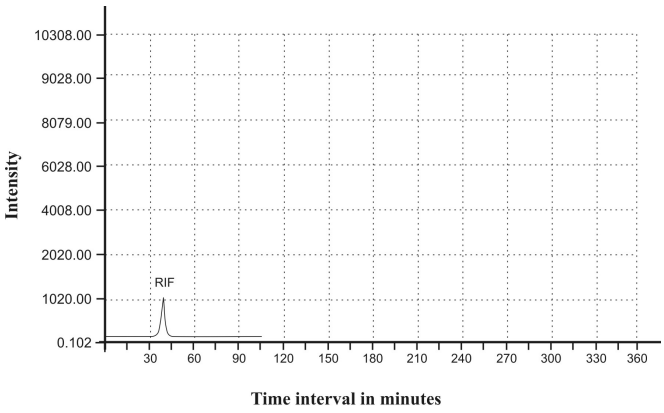


Figure-10

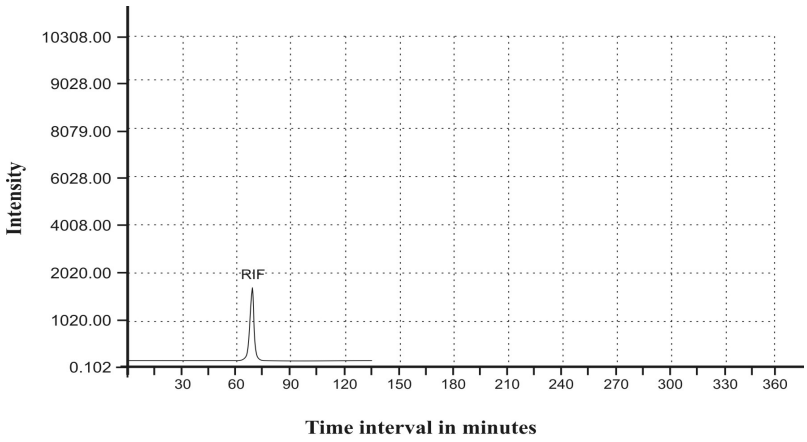
IN-VIVO PHARMACOKINETIC STUDY

HPLC Graphs of Rifampicin alone



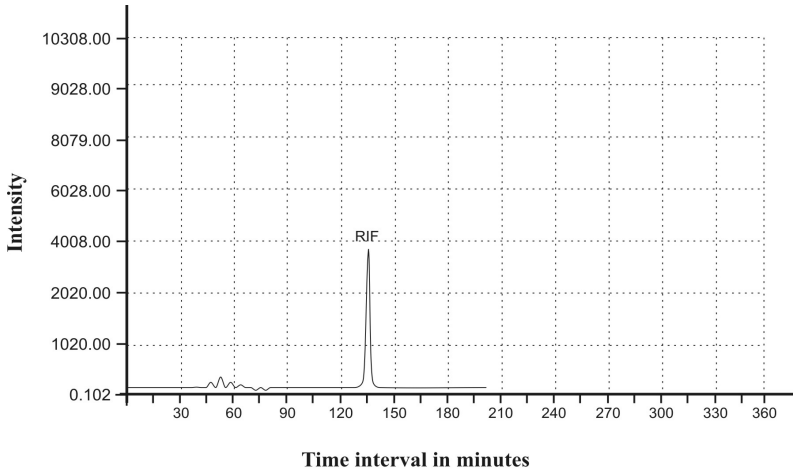
PLASMA SAMPLE AT 30 MIN.

Fig- 11



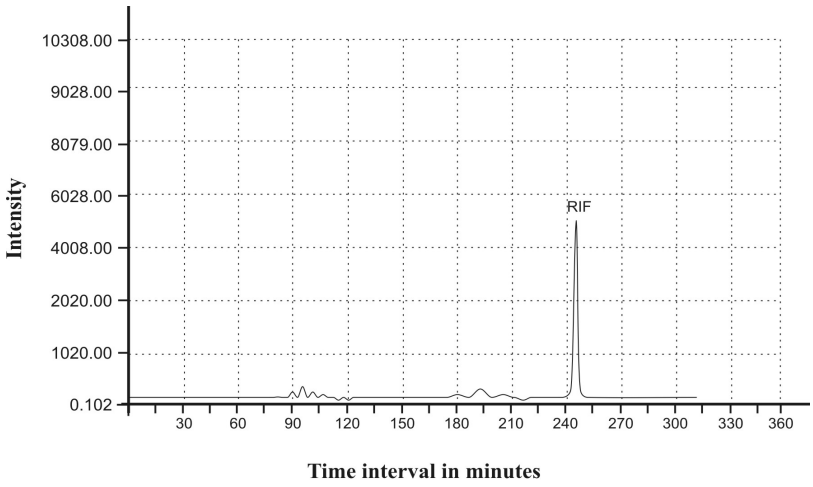
PLASMA SAMPLE AT 60 MIN.

Fig-12



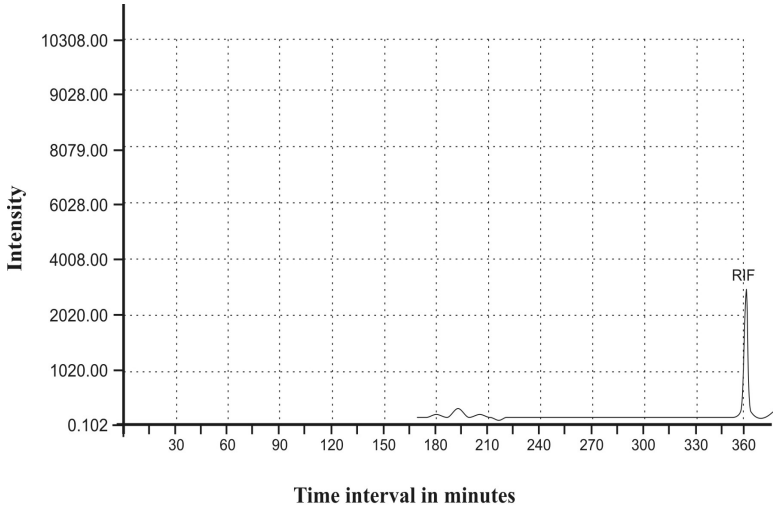
PLASMA SAMPLE AT 120 MIN.

Fig-13



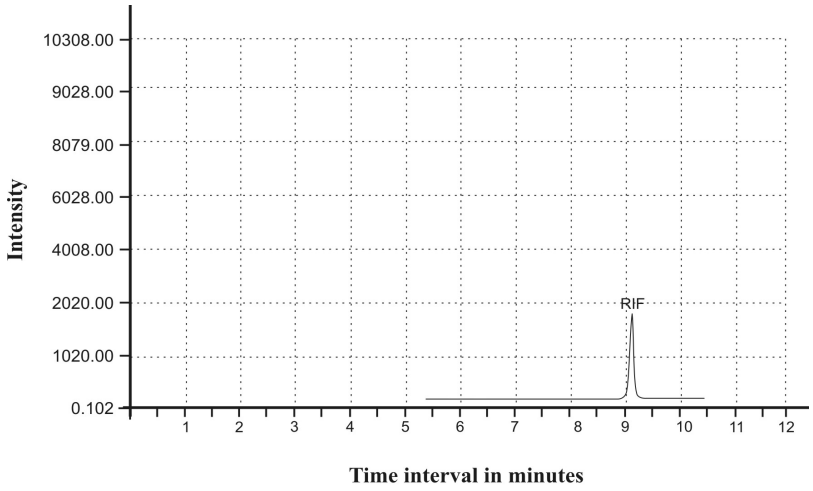
PLASMA SAMPLE AT 240 MIN.

Fig-14



PLASMA SAMPLE AT 360 MIN.

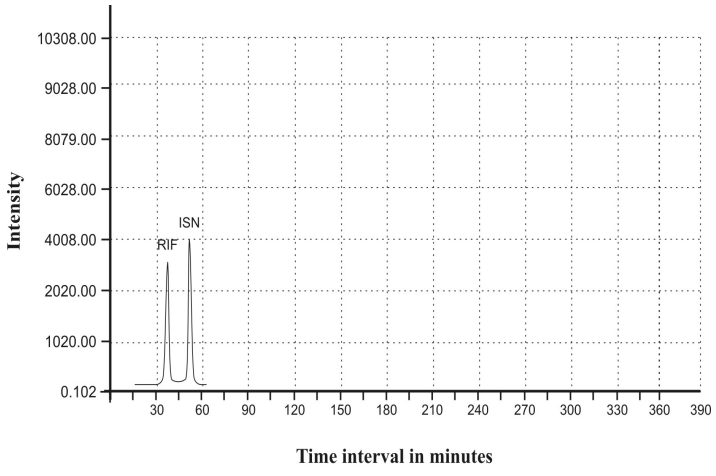
Fig-15



PLASMA SAMPLE AT 9 HRS.

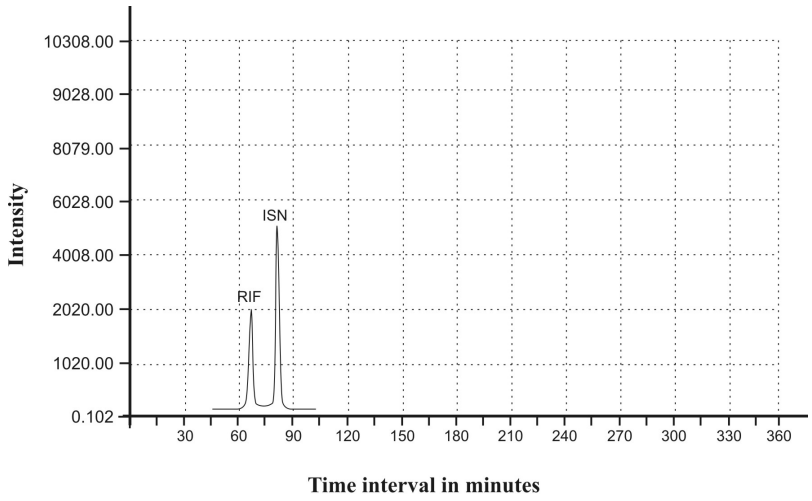
Fig-16

HPLC Graphs of Rifampicin+ Isoniazid



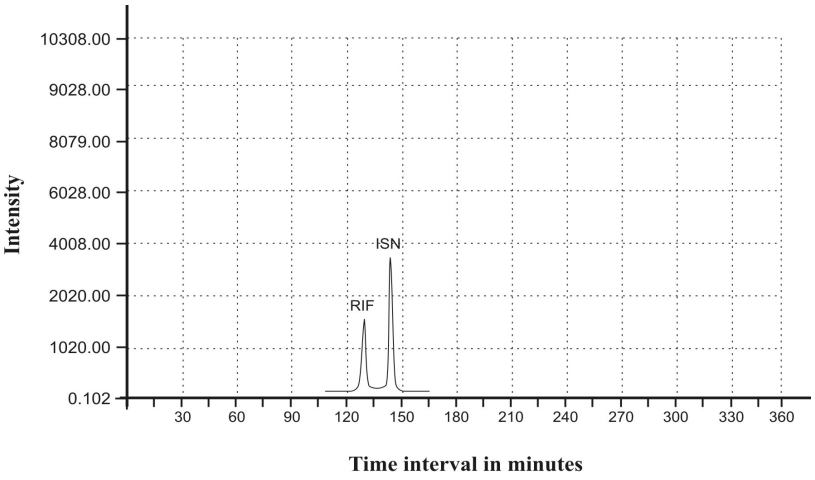
PLASMA SAMPLE AT 30 MIN.

Fig-17



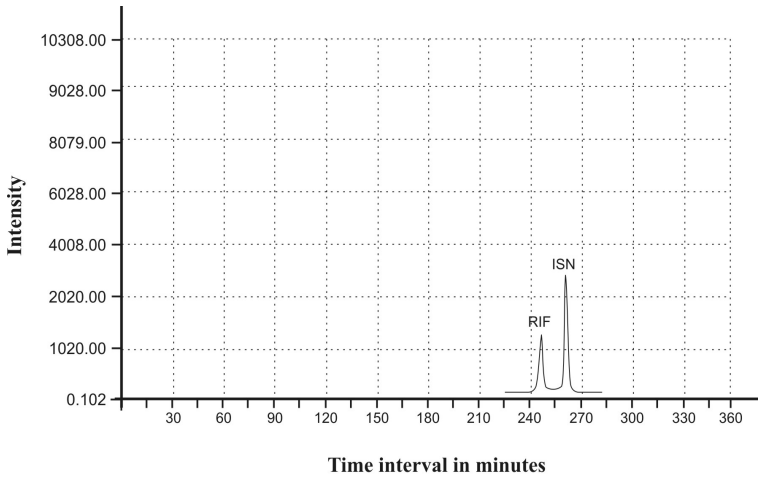
PLASMA SAMPLE AT 60 MIN.

Fig-18



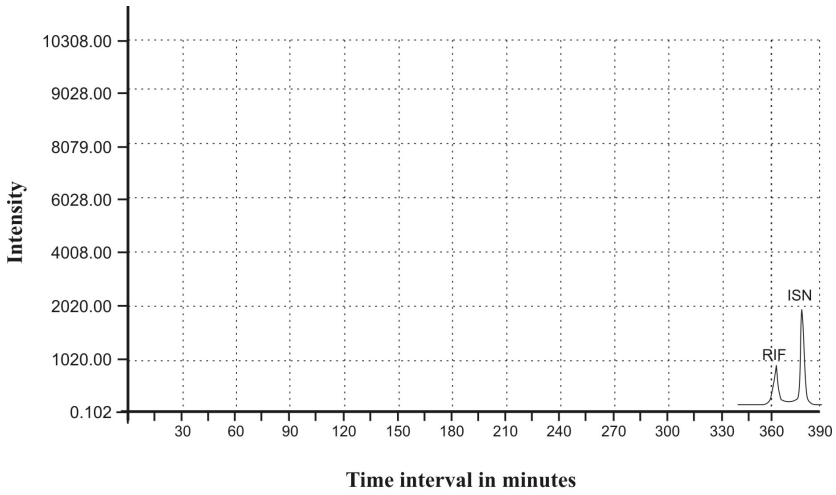
PLASMA SAMPLE AT 120 MIN.

Fig-19



PLASMA SAMPLE AT 240 MIN.

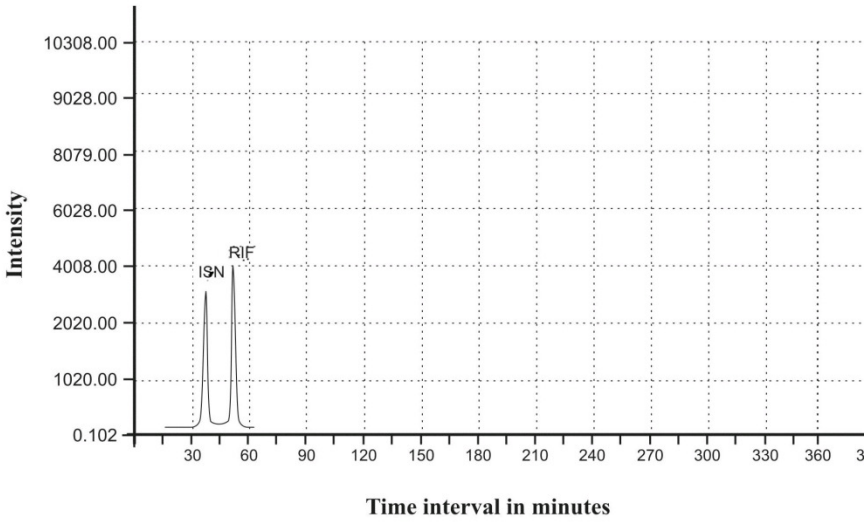
Fig-20



PLASMA SAMPLE AT 360 MIN.

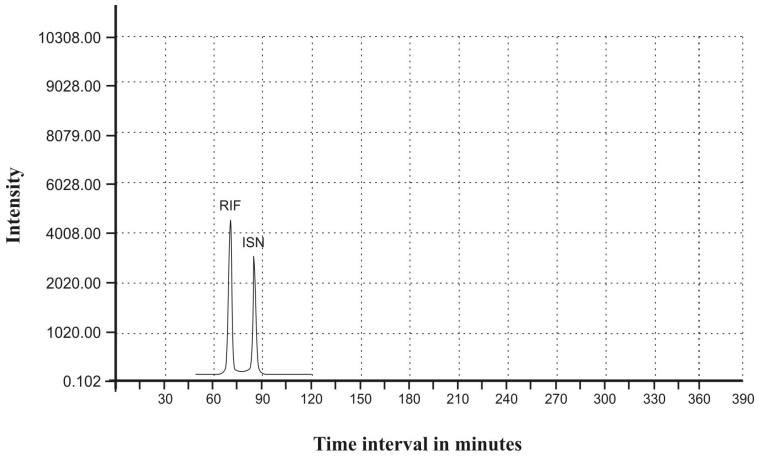
Fig-21

HPLC Graphs of Rifampicin +Isoniazid +Ascorbic acid



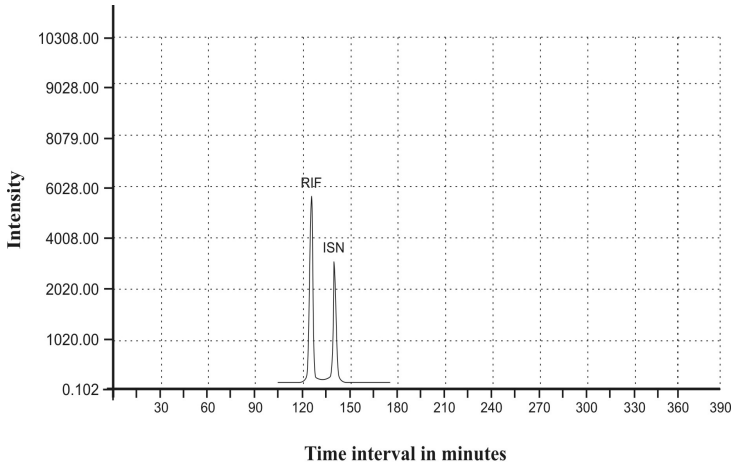
PLASMA SAMPLE AT 30 MIN

Fig-22



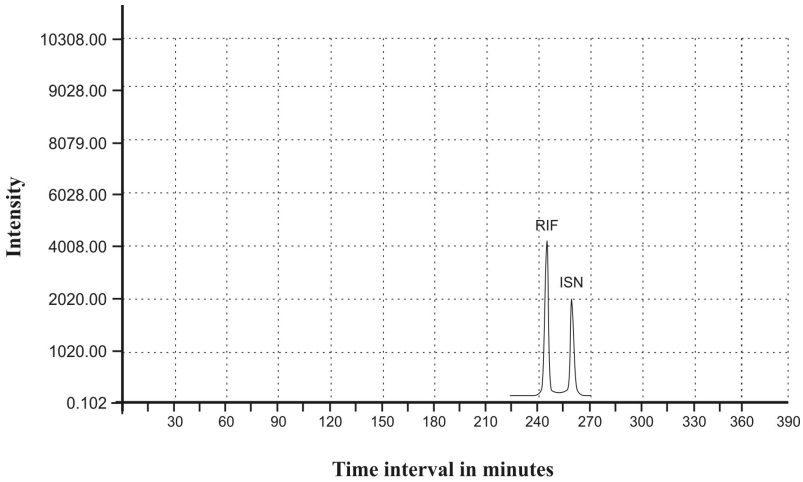
PLASMA SAMPLE AT 60 MIN.

Fig-23



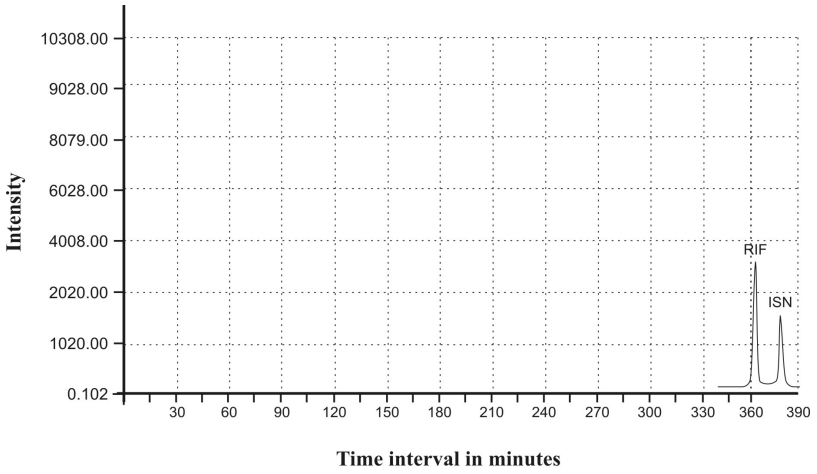
PLASMA SAMPLE AT 120 MIN.

Fig-24



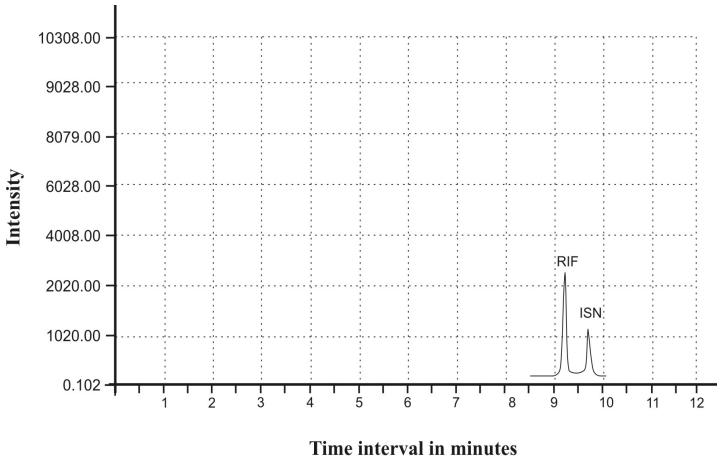
PLASMA SAMPLE AT 240 MIN.

Fig-25



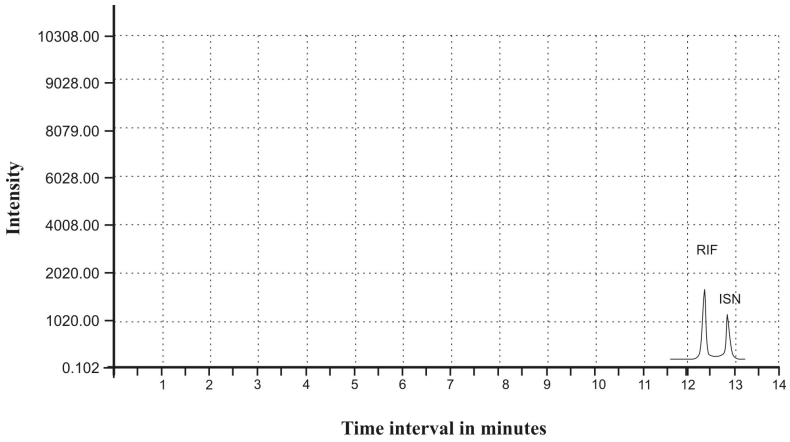
PLASMA SAMPLE AT 360 MIN.

Fig-26



PLASMA SAMPLE AT 9 HRS.

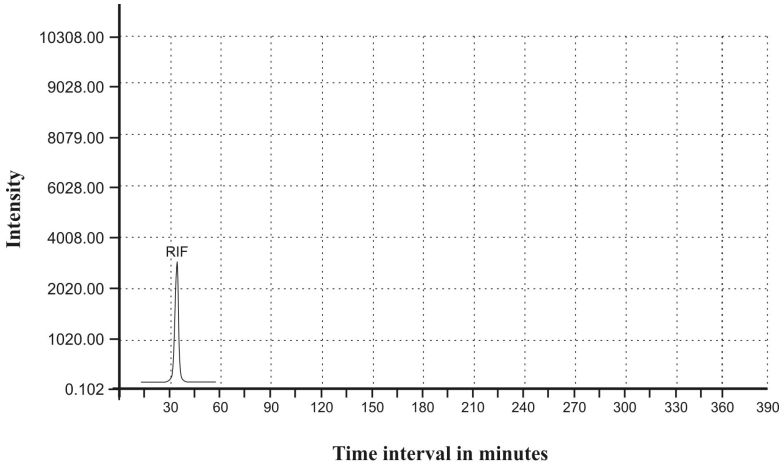
Fig-27



PLASMA SAMPLE AT 12 HRS.

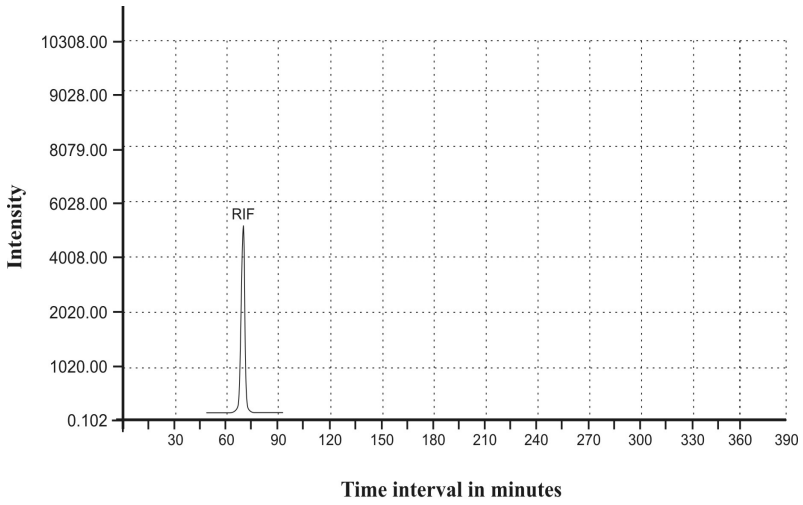
Fig-28

HPLC Graphs of Rifampicin +Ascorbic acid



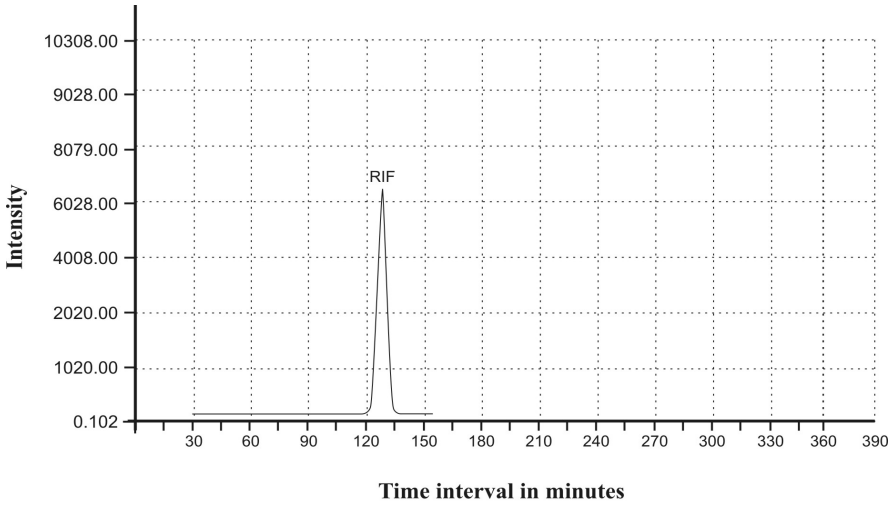
PLASMA SAMPLE AT 30 MIN.

Fig-29



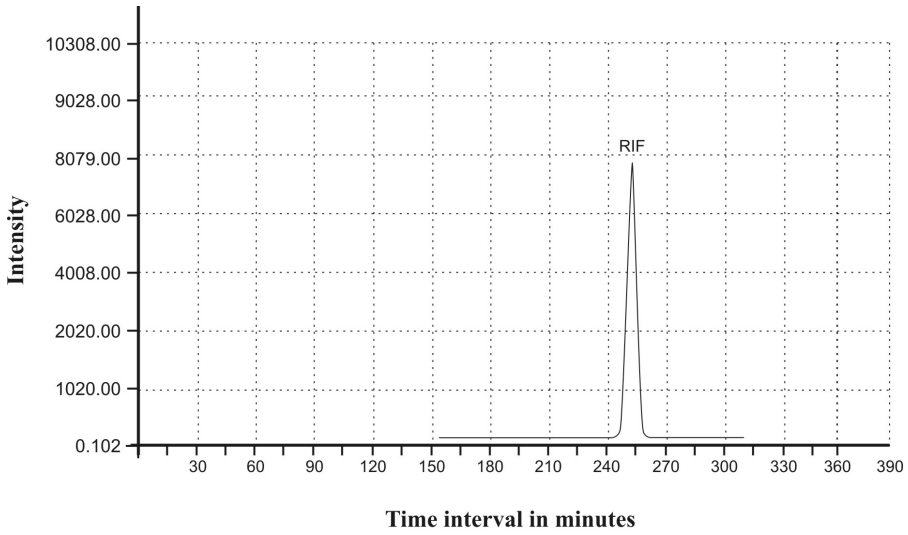
PLASMA SAMPLE AT 60 MIN.

Fig-30



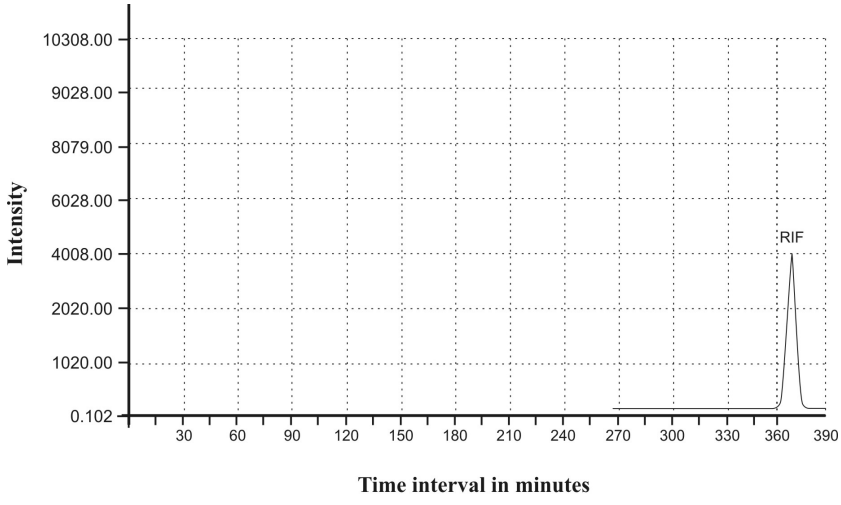
PLASMA SAMPLE AT 120 MIN.

Fig-31



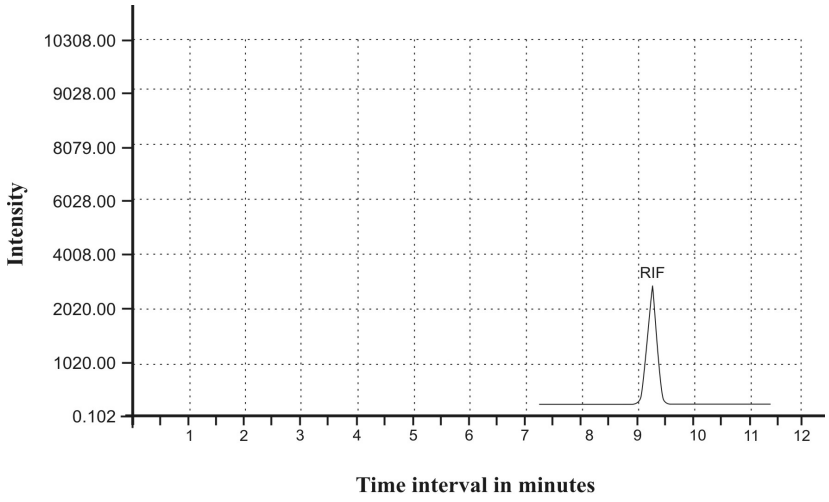
PLASMA SAMPLE AT 240 MIN.

Fig-32



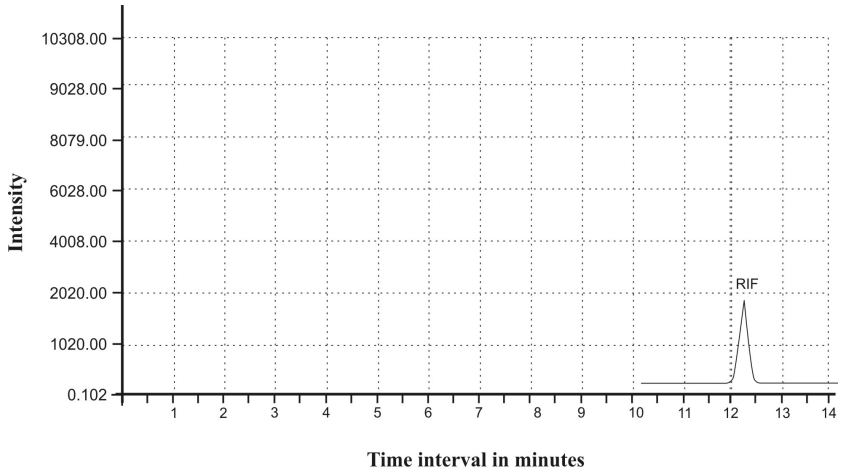
PLASMA SAMPLE AT 360 MIN.

Fig-33



PLASMA SAMPLE AT 9 HRS.

Fig-34



PLASMA SAMPLE AT 12 HRS.

Fig-35

Table25.

PLASMA CONCENTRATION OF RIFAMPICIN ALONE AT VARIOUS TIME INTERVALS IN GROUP1 RABBITS

Time(hr)	Animals			Mean± S.E.M
	1	2	3	
0.5	3.9	4	3.7	3.8±0.088
1	5	5.2	5.4	5.2±0.1155
2	6.8	7.1	7.5	7.1±0.2028
4	5.6	6.2	5.7	5.8±0.1856
6	3.7	3.5	3.2	3.4±0.1453
9	0.9	1	1.1	1±0.05774
12	-	-	-	-

Table26.

PLASMA CONCENTRATION OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID AT VARIOUS TIME INTERVALS IN GROUP2 RABBITS

Time(hr)	Animals			Mean± S.E.M
	1	2	3	
0.5	1.9	2.3	2.6	2.2±0.202
1	4	4.2	4.4	4.2±0.1155
2	5.1	5.3	4.9	5.1±0.115
4	3.9	4	4.1	4±0.057
6	2.9	3	2.8	2.9±0.054
9	-	-	-	-
12	-	-	-	-

Table27.

PLASMA CONCENTRATIONS OF RIFAMPICIN IN THE PRESENCE OF ISONIAZID + ASCORBIC ACID AT VARIOUS AT VARIOUS TIME INTERVALS IN GROUP3 RABBITS

Time(hr)	Animals			Mean±S.E.M
	1	2	3	
0.5	5.4	5.8	6	5.7±0.176
1	6.9	7.1	7.4	7.1±0.145
2	9	9.1	9.2	9.1±0.057
4	8.6	8	8.8	8.4±0.24
6	6.7	6.3	6.6	6.5±0.12
9	2.8	2.3	2.7	2.6±0.152
12	1.6	2.1	1.9	1.8±0.145

Table28.

Time(hr)	Animals			Mean±S.E.M
	1	2	3	
0.5	6	6.2	6.4	6.2±0.115
1	8.1	8.6	8.4	8.3±0.1453
2	9.7	10.1	10.3	10±0.1764
4	9.9	10.2	9.8	9.9±0.01202
6	8	8.1	7.9	8±0.0574
9	4.1	4.3	3.9	4.1±0.1155
12	2.7	1.9	2.3	2.3±0.2309

PLASMA CONCENTRATION OF RIFAMPICIN IN THE PRESENCE OF ASCORBIC ACID AT VARIOUS TIME INTERVALS IN GROUP4 RABBITS

PHARMACOKINETIC CALCULATIONS

Table29.

S.no	Parameters	Animals			Mean±S.E.M
		1	2	3	
1	K_{ehr}^{-1}	0.398	0.335	0.358	0.363±0.0184
2	K_{ahr}^{-1}	0.419	0.425	0.431	0.425±0.0034
3	$T_{1/2hr}$	1.7	1.85	1.92	1.823±0.064
4	$V_{d\ lit}$	0.91	0.93	0.95	0.93±0.011
5	$T_{max\ hr}$	1.2	1.3	1.5	1.333±0.088
6	$C_{max\ \mu g/ml}$	5.90	5.82	6	5.906±0.052
7	$AUC_{0\text{-}12\mu g/ml\ ,h}$	34.31	36.21	31.36	33.96±1.411
8	$AUC_{0\text{-}\infty 12\mu g/ml\ ,h}$	60.61	62.60	59.94	61.05±0.798

PHARMACOKINETICS OF RIFAMPICIN ALONE IN GROUP 1(N=3).

Table30.

PHARMACOKINETICS OF RIFAMPICIN + ISONIAZID IN GROUP 2 (N=3)

S.no	Parameters	Animals			Mean±S.E.M
		1	2	3	
1	Ke_{hr}^{-1}	0.295	0.299	0.310	0.301±0.004
2	K_{ahr}^{-1}	0.3151	0.318	0.321	0.318±0.001
3	$T_{1/2hr}$	2.7	2.4	2.5	2.533±0.088
4	$V_{d\ lit}$	0.83	0.87	0.89	0.863±0.017
5	$T_{max\ hr}$	1.61	1.63	1.68	1.64±0.020
6	$C_{max\ \mu g/ml}$	4.10	4.16	4	4.086±0.046
7	$AUC_{0\text{-}12\mu g/ml.h}$	29.13	28.16	26.15	27.81±0.877
8	$AUC_{0\text{-}\infty\mu g/ml.h}$	50.14	54.31	56.14	53.53±1.775

Table31.

PHARMACOKINETICS OF RIFAMPICIN +ISONIAZID +ASCORBIC ACID IN GROUP 3 (N=3)

S.no	Parameters	Animals			Mean±S.E.M
		1	2	3	
1	Ke_{hr}^{-1}	0.418	0.421	0.433	0.424±0.004
2	K_{ahr}^{-1}	0.525	0.533	0.545	0.534±0.005
3	$T_{1/2hr}$	0.99	0.97	1	1.594±0.008
4	$V_{d\ lit}$	0.99	1	1.2	1.603±0.068
5	$T_{max\ hr}$	1	1.1	1.3	1.133±0.088
6	$C_{max\ \mu g/ml}$	9.24	9.28	9.31	9.276±0.020
7	$AUC_{0\text{-}12\mu g/mlh}$	64.68	68.16	74.6	69.146±2.906
8	$AUC_{0\text{-}\infty\mu g/mlh}$	78.90	81.77	86.7	82.48±2.099

Table32.

PHARMACOKINETICS OF RIFAMPICIN +ASCORBIC ACID IN GROUP 4 (N=3).

S.no	Parameters	Animals			Mean±S.E.M
		1	2	3	
1	Ke_{hr}^{-1}	0.615	0.614	0.616	0.518±.006
2	K_{ahr}^{-1}	0.514	0.511	0.531	0.615±0.0005
3	$T_{1/2hr}$	0.95	0.97	0.94	0.953±0.008
4	$V_{d\ lit}$	1	1.3	1.5	1.266±0.145
5	$T_{max\ hr}$	1.2	1.4	1.6	1.02±0.115
6	$C_{max\ \mu g/ml}$	10	10.2	10.4	10.2±0.115
7	$AUC_{0\text{-}12\mu g/mlh}$	70.81	72.03	71.42	71.42±0.352
8	$AUC_{0\text{-}\infty\mu g/mlh}$	77.90	86.77	81.10	89.54±2.593

GRAPHICAL REPRESENTATION OF PHARMACOKNETICS PARAMETERS

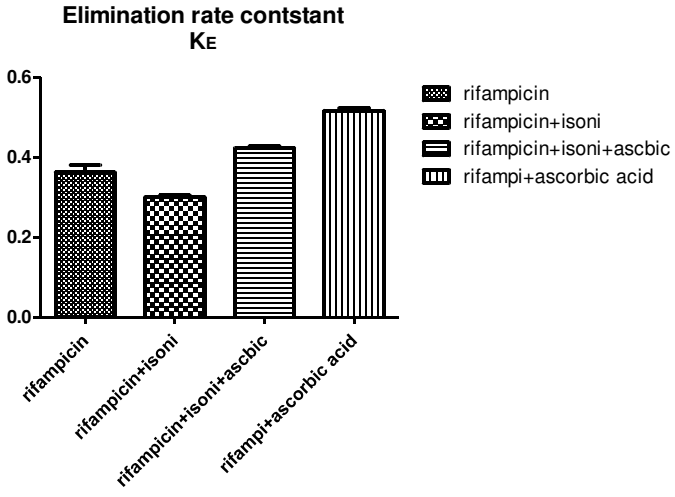


Fig-36

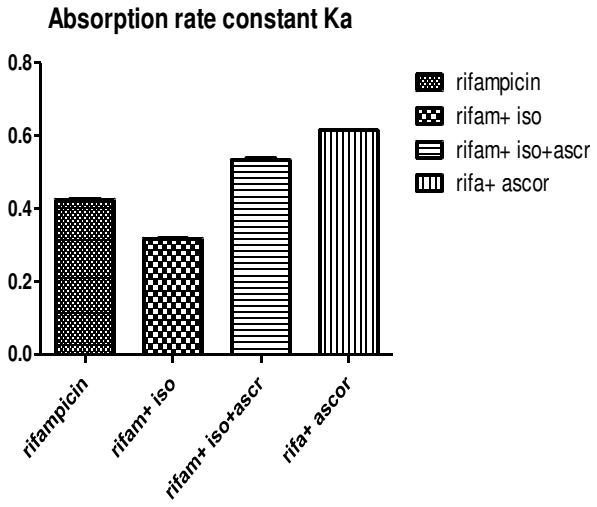

Absorption rate constant Ka
0.8
0.6
0.4
0.2
0.0
rifampicin
rifam+ iso
rifam+ iso+ascr
rifa+ ascor
rifampicin
rifam+ iso
rifam+ iso+ascr
rifa+ ascor

Fig-37

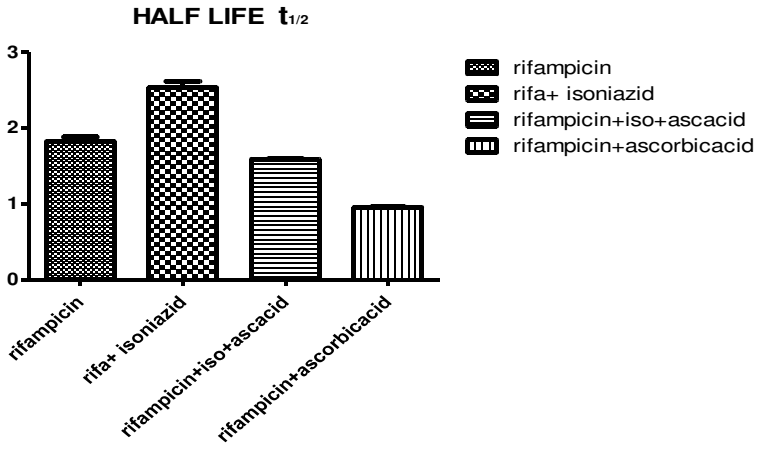

HALF LIFE t1/2
3
2
1
0
rifampicin
rifa+ isoniazid
rifampicin+iso+ascacid
rifampicin+ascorbicacid
rifampicin
rifa+ isoniazid
rifampicin+iso+ascacid
rifampicin+ascorbicacid

Fig-38

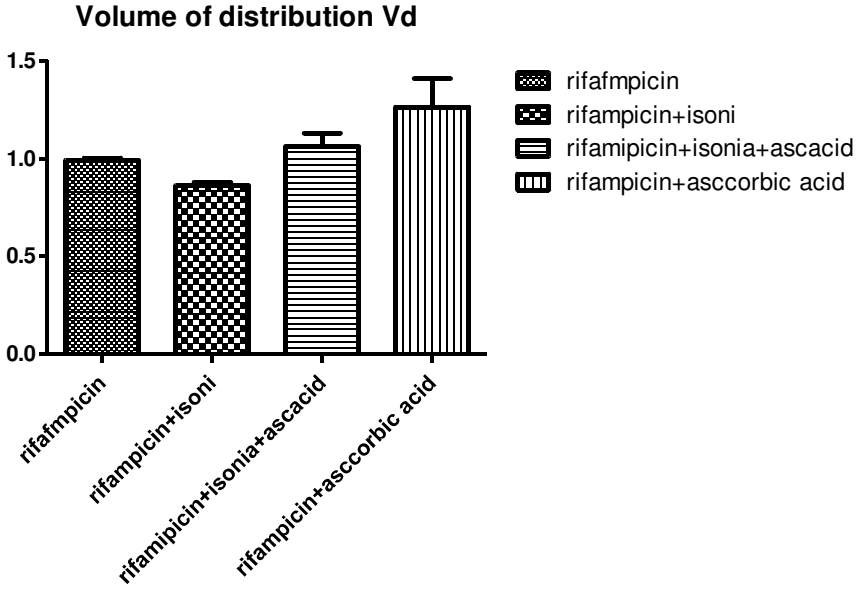

Volume of distribution Vd
1.5
1.0
0.5
0.0
rifafmpicin
rifampicin+isoni
rifamipicin+isonia+ascacid
rifampicin+asccorbic acid
rifafmpicin
rifampicin+isoni
rifamipicin+isonia+ascacid
rifampicin+asccorbic acid

Fig-39

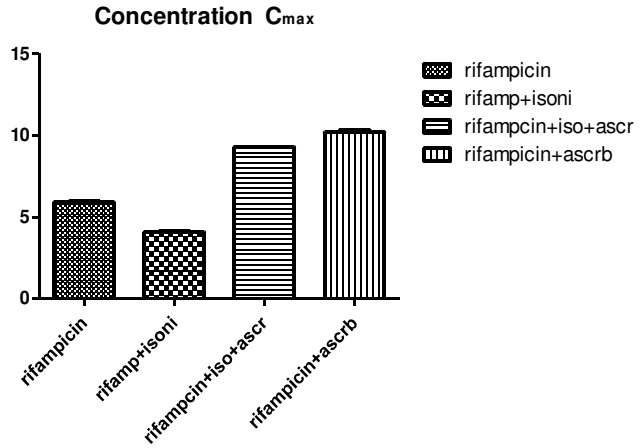

Concentration Cmax
15
10
5
0
rifampicin
rifamp+isoni
rifampcin+iso+ascr
rifampicin+ascrb
rifampicin
rifamp+isoni
rifampcin+iso+ascr
rifampicin+ascrb

Fig-40

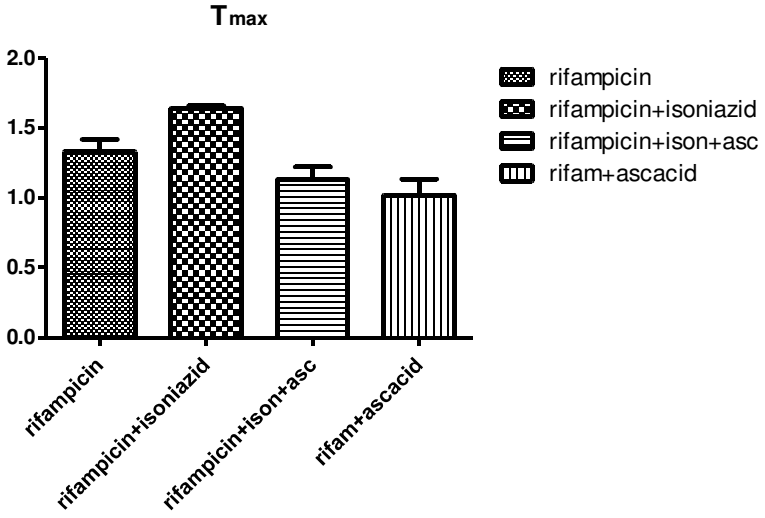

Tmax
2.0
1.5
1.0
0.5
0.0
rifampicin
rifampicin+isoniazid
rifampicin+ison+asc
rifam+ascacid
rifampicin
rifampicin+isoniazid
rifampicin+ison+asc
rifam+ascacid

Fig-41

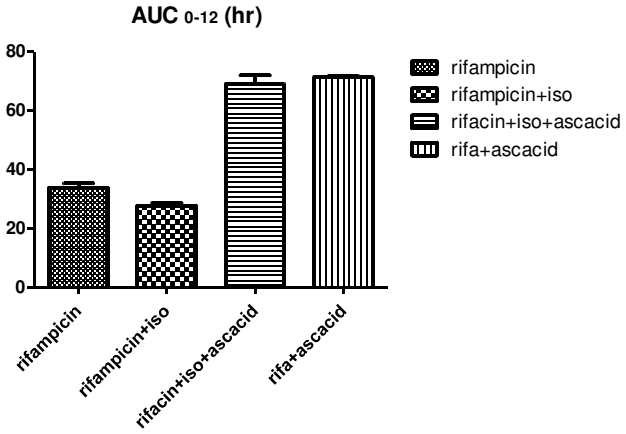

AUC 0-12 (hr)
80
60
40
20
0
rifampicin
rifampicin+iso
rifacin+iso+ascacid
rifa+ascacid
rifampicin
rifampicin+iso
rifacin+iso+ascacid
rifa+ascacid

Fig-42

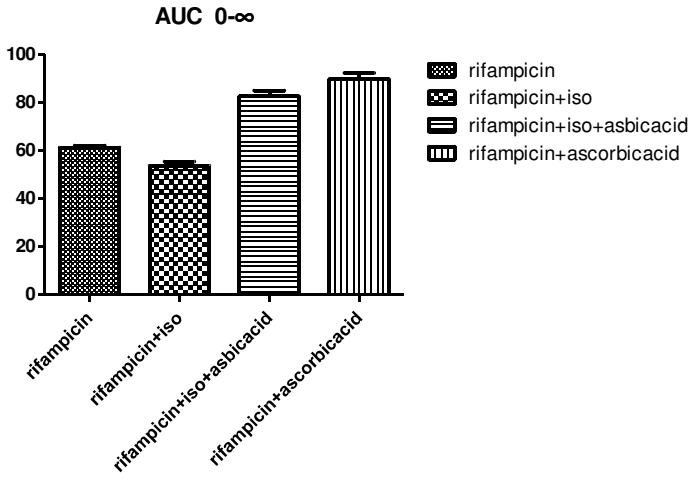


Fig-43

IN-VITRO DISSOLUTION STABILITY STUDY

The results of in-vitro dissolution stability of RIF, RIF+INH, RIF +Ascorbic acid (varying concentrations 125mg,250mg,and500mg) and RIF+ Ascorbic acid (varying concentrations 125mg,250mg,and500mg) are shown in tables9-16 and figure9. The stability of rifampicin was ascertained from the % release of the drug at 60min, as rifampicin was absorbed maximal with in an hour from the acidic environment of the stomach and so the % release of drug was limited to 60min. The % drug release of rifampicin in p^H1.2 buffer at 60min was57.2% and it was reduced to 26% in the presence of isoniazid and this reduction was statically significant (***$p<0.001$).

Ascorbic acid in (varying concentrations 125mg, 250mg, and500mg) reverse the % drug release and the maximum effect was observed (74.4%) with higher concentration of ascorbic acid (500mg) and this reduction was statically significant (***p<0.001), this finding indicates that ascorbic acid protects rifampicin degradation by isoniazid and so an enhanced amount of rifampicin release was observed in the presence of ascorbic acid. The effect of ascorbic acid on % release of rifampicin alone at 60min was also observed and it was found the % release of rifampicin increased as the concentration of ascorbic acid increased and this increase was found to be ascorbic acid concentration dependent. Maximum % release of rifampicin (79.6%) was observed with higher concentration of ascorbic acid (500mg). The above findings clearly suggest and improved % release of rifampicin either alone (or) in the presence of isoniazid by the effect of ascorbic acid. The results of the dissolution study reveal that absorption of rifampicin even in the presence of isoniazid can be significantly improved through inhibition of degradation of rifampicin with more availability of rifampicin for dissolution and absorption.

From the in-vitro release data the % degradation of rifampicin at 60min was also determined and the influence of ascorbic acid on stabilizing rifampicin alone (or) in the presence of isoniazid was ascertained. The results of % degradation of rifampicin are shown in tables17-24 and figure10, rifampicin degraded to the extent 42.7 % at p^H 1.2 buffer at 60min and the of % degradation of rifampicin significantly increased to (74 %) in the presence of isoniazid (***p<0.001). As ascorbic acid (125mg) addition reduce the degradation of rifampicin from 42.7%-32.1% (***p<0.001) and as the concentration of ascorbic acid increased the % degradation of rifampicin was proportionately decreased , the % reduction of degradation of rifampicin in the presence of ascorbic acid 1254mg, 250mg, and 500mg was 32.1%, 24.5%, and 20.3% respectively and this reduction was statically significant (***p<0.001).

Similarly effects of ascorbic acid were observed on the % degradation of rifampicin in the presence of isoniazid, the % degradation of rifampicin in the presence of isoniazid was 74% and degradation of rifampicin was significantly reduced (***p<0.001) to 35.8%, 29.2%, and 25.5% with ascorbic acid 125mg, 250mg, and 500mg respectively,

these data signal the beneficial effects of ascorbic acid addition to overcome (or) minimize the degradation of rifampicin in the gastric environment.

In-vivo pharmacokinetic study:

In-vivo pharmacokinetic parameters like K_e, K_a, T_{max}, C_{max}, V_d, $T_{1/2}$, AUC^{0-12}, $AUC_{0-\infty}$ there also studied in rabbits in order to ascertain whether the in-vitro release and degradation data of rifampicin would be truly reflected in-vivo bioavailability. The results of the pharmacokinetic study as shown in tables 25-32 and figures 11-43.

K_e, K_a, C_{max}, AUC^{0-12},$AUC_{0-\infty}$ of rifampicin were significantly increased by the effect of ascorbic acid (***p<0.001), these findings clearly suggest that the amount of rifampicin available for absorption was significantly increased in the presence of ascorbic acid due the effect of ascorbic acid on protecting the rifampicin against degradation in the acidic environment and as such K_e, K_a, C_{max}, AUC^{0-12},$AUC_{0-\infty}$ were significantly increased, isoniazid significantly reduced K_e, K_a, C_{max}, AUC^{0-12},$AUC_{0-\infty}$ of rifampicin (***p<0.001), indicative of the catalytic degradation effect of isoniazid on rifampicin which is inconsistent we early finding.[49, 50, 51, 52, 53] As ascorbic acid reversed the above pharmacokinetic parameters of rifampicin brought about by isoniazid to values greater than the corresponding values, pure rifampicin and the effect was statically significant (***p<0.001), these finding again support the contention that ascorbic acid protects rifampicin against degradation even in the presence of isoniazid, As K_e, K_a of rifampicin alone (or) in the presence of isoniazid were improved by ascorbic acid, the $t_{1/2}$,and t_{max} were significantly reduced acid (***p<0.001).

Previously it has been documented that ascorbic acid was used stabilize rifampicin in the plasma sample. However to this effect, to the best of our knowledge no report is available on the effect of ascorbic acid on the in-vitro stability and in-vivo pharmacokinetics of rifampicin, this is first time we are reporting on the beneficial effects of ascorbic acid in preventing (or) minimizing rifampicin degradation on improving its bioavailability.

Our findings in the present study propose that degradation of rifampicin in combination with isoniazid (or) through fixed dose combinations of rifampicin, isoniazid, pyrazinamide, and ethambutol can be controlled by using ascorbic acid in appropriate concentrations. It has been documented that tuberculosis patients should be given ascorbic acid 1000mg/day and therefore our study justifies the recommendation of ascorbic acid addition to rifampicin formulations in order to control degradation and improve bioavailability of rifampicin for effective management of tuberculosis. It strongly recommended for the clinical study on the viability of beneficial effects of improved bioavailability of rifampicin in clinical practice.

SUMMARY AND CONCLUSION

9. SUMMARY AND CONCLUSION

- Rifampicin degrades in pH 1.2 medium.
- The degradation of rifampicin was further influenced by isoniazid.
- Ascorbic acid in varying concentrations (125mg, 250mg and 500mg) can control the degradation of rifampicin even in the presence of isoniazid.
- The stabilization effect of ascorbic acid on rifampicin was reflected in the pharmacokinetic parameters.
- K_a. K_e, C_{max}, AUC_{0-12}, $AUC_{0-\infty}$ of rifampicin were significantly increased by the effect of ascorbic acid.
- $T_{1/2}$, and T_{max} of rifampicin were also significantly reduced by ascorbic acid.
- It can be concluded that ascorbic acid can be included in the formulations containing rifampicin for improving its bioavailability.

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